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ABSTRACT

Included are four activity units: (1) Measuring Dissolved Oxygen Quantitatively; (2) Collecting and Cultivating Marine Bacteria; (3) To Recognize, Record, and Analyze Characteristics of a Sandy Beach Environment; and (4) Quantitative and Qualitative Analysis of Phosphate in Water. All the activities are designed to be used by secondary school students. Each activity includes student instructions, lists of materials needed, selected references, and supplementary activities. (RH)

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307

ED141160

MEASURING DISSOLVED OXYGEN QUANTITATIVELY

A Learning Experience for
Coastal and Oceanic
Awareness Studies

Produced by

MARINE ENVIRONMENT CURRICULUM STUDY
MARINE ADVISORY SERVICE
UNIVERSITY OF DELAWARE

and

POPULATION-ENVIRONMENT CURRICULUM STUDY
COLLEGE OF EDUCATION
UNIVERSITY OF DELAWARE

as part of a

PLAN FOR ENVIRONMENTAL EDUCATION

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Please send evaluations

of learning experiences

to

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310 Willard Hall Education Building

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Newark, Delaware 19711

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TITLE: MEASURING DISSOLVED OXYGEN QUANTITATIVELY

* CONCEPT: V.B.2.b.(2)

V. The activities of human populations may degrade the environment and restrict the quality of human life.

B. Human activities cause pollution.

2. Pollution reduces the quality and usefulness of the environment.

b. Reduced water quality affects many human activities.

(2) THE QUALITY OF WATER IS AFFECTED BY ITS INDUSTRIAL, AGRICULTURAL AND DOMESTIC USES.

** MARINE CONCEPT: 1.23

1. An abundance of water makes the earth unique in our solar system.

1.2 Water in the environment contains a variety of substances in suspension and in solution.

1.23 WATER IN NATURE USUALLY CONTAINS GASES, ORGANIC COMPOUNDS, AND MINERAL SALTS.

GRADE LEVEL: 10-12

SUBJECTS: Biology, Chemistry

CLASS PERIODS: Various

AUTHORS: Cross, Donofrio, and Levitan

* From A Conceptual Scheme for Population-Environment Studies, 1973. Cost $2.50.

** From Marine Environment Proposed Conceptual Scheme, 1973. No charge.

Both conceptual schemes are available from Robert W. Stegner, Population-Environment Curriculum Study, 310 Willard Hall, University of Delaware, Newark, DE 19711.

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INTRODUCTION

The concentration of dissolved oxygen in an aquatic environment is a significant measure of its quality--its ability to support life. Almost all living things require oxygen (some bacteria are exceptions). The amount of oxygen required by each organism may differ, but most organisms need at least a small amount. Hence, the amount of dissolved oxygen in water affects the presence and health of organisms living there.

Using the Delaware River as an example, let's see what causes oxygen to disappear from natural water systems. The Delaware River contains large amounts of decaying matter, the main sources of which are municipal and industrial wastes. In the process of decay, bacteria use the oxygen to break down organic matter into simpler forms such as nitrates, phosphates, sulfates and carbon dioxide. These products can then be used by plants in photosynthesis. However, because the process of decay uses oxygen, it can lead to depletion of dissolved oxygen in the water. When this occurs, anaerobic forms of bacteria predominate and the final product of decomposition is ammonia. Plants are unable to use the ammonia. Deprived of the necessary nutrients provided by aerobic bacterial breakdown, the plants die. And without oxygen present, animals are unable to live in that area.

In the oceans, phytoplankton provide most of the dissolved oxygen, just as they provide most of the atmospheric oxygen. Thus, the dissolved oxygen content of water depends to a large extent on factors that support phytoplankton. These factors include light and the availability of nutrients, such as nitrates and phosphates.

BACKGROUND INFORMATION

The dissolved oxygen (abbreviated DO) content of water refers to the amount of oxygen which is available for use by aquatic life. Because of oxygen's importance to the survival of organisms, a dissolved oxygen test is usually included in any type of water survey, including environmental impact studies.

The amount of DO varies with the properties of the water being sampled. Temperature, salinity and organic content of the water have the greatest effects on DO content. Higher water temperature or increased salinity results in decreased DO (see Table 1, p. 3). Because the decay process consumes oxygen, an increase in organic content (from pollution for example) results in a decrease in DO. Therefore, a low DO content would indicate low water quality and an alteration in the type of organism normally found in the water may occur.

Dissolved oxygen content in water is usually measured quantitatively by means of the Winkler-Azide titration method. In this learning experience, the method will be used to determine the DO content in water and the manner in which it varies with:

1) temperature
2) salinity
3) organic matter content

DO vs. organic matter content will then be used to demonstrate the impact of organic pollution on water quality.

Table 1: SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR

Temperature in °C	Chloride Concentration in Water-mg/liter				
	0	5,000	10,000	15,000	20,000
	Dissolved Oxygen, in mg/liter				
0	14.6	13.8	13.0	12.1	11.3
1	14.2	13.4	12.6	11.8	11.0
2	13.8	13.1	12.3	11.5	10.8
3	13.5	12.7	12.0	11.2	10.5
4	13.1	12.4	11.7	11.0	10.3
5	12.8	12.1	11.4	10.7	10.0
6	12.5	11.8	11.1	10.5	9.8
7	12.2	11.5	10.9	10.2	9.6
8	11.9	11.2	10.6	10.0	9.4
9	11.6	11.0	10.4	9.8	9.2
10	11.3	10.7	10.1	9.6	9.0
11	11.1	10.5	9.9	9.4	8.8
12	10.8	10.3	9.7	9.2	8.6
13	10.6	10.1	9.5	9.0	8.5
14	10.4	9.9	9.3	8.8	8.3
15	10.2	9.7	9.1	8.6	8.1
16	10.0	9.5	9.0	8.5	8.0
17	9.7	9.3	8.8	8.3	7.8
18	9.5	9.1	8.6	8.2	7.7
19	9.4	8.9	8.5	8.0	7.6
20	9.2	8.7	8.3	7.9	7.4
21	9.0	8.6	8.1	7.7	7.3
22	8.8	8.4	8.0	7.6	7.1
23	8.7	8.3	7.9	7.4	7.0
24	8.5	8.1	7.7	7.3	6.9
25	8.4	8.0	7.6	7.2	6.7

Taken from Taras, M. J. et al.,eds. 1971. Standard Methods for the Examination of Water and Wastewater (13th ed.). American Public Health Assoc., New York, p. 480. Used with permission of the publisher.

NOTE TO THE TEACHER:

This lab exercise may require two lab periods. To facilitate the lab, all reagents, water samples and lab set-ups could be prepared beforehand. However, this exercise may also serve as a lesson about the preparation of reagents or be used in conjunction with a lesson on normality and titration techniques.

If you do not want to make up the solutions and reagents, there is a dissolved oxygen kit available from Hach Chemical Company. In this kit, Hach modifies the standard Winkler method. There are enough reagents for 100 tests. The kit sells for about $20.50 and can be ordered under the title: Dissolved Oxygen Test Kit, Model OX-2P from

Hach Chemical Company
P.O. Box 907
Ames, Iowa 50010

Portions of the lab exercise may be omitted. The most important part of the lab is to perform a Winkler titration in a normal tap water sample so students become familiar with the method, then perform a titration on an organically polluted water sample. This is important in understanding the effects of pollution on water quality and aquatic life.

A more elementary qualitative approach to dissolved oxygen is presented in learning experience V.B.2.b.(2) (Mar. 1.23) "Dissolved Oxygen Measured Qualitatively," available from Population-Environment Curriculum Study, 310 Willard Hall Education Building, University of Delaware, Newark, DE 19711.

THEORY

Because dissolved oxygen is sensitive to agitation and temperature, sampling procedures must be designed to avoid aeration and warming. Furthermore, since biological and chemical processes may be occurring in samples taken from natural water systems, the test must either be done immediately or the dissolved oxygen must be fixed. In the Winkler-Azide method, dissolved oxygen is fixed by the addition of manganese sulfate ($MnSO_4$) and an alkali-iodide-azide reagent ($NaOH$-KI-NaN_3). The azide (NaN_3) is used to eliminate interference by nitrite ions.

There are five reactions:

$$2MnSO_4 + 4NaOH + O_2 \longrightarrow 2MnO(OH)_2 + 2Na_2SO_4 \qquad (1)$$

This is the reaction which fixes the dissolved oxygen in the form of $MnO(OH)_2$. A brown precipitate should appear if DO is present. Upon addition of H_2SO_4 to cause an acidic pH, two reactions occur:

$MnO(OH)_2$ oxidation of iodide ions (I^-) to provide free iodine (I_2):

a. $$2MnO(OH) + 2KI + 2H_2SO_4 \longrightarrow 2MnSO_4 + K_2SO_4 + I_2 + 4H_2O \qquad (2)$$

and Azide destruction of interfering nitrite (NO_2^-):

b. $$NaN_3 + H^+ \text{ (acid)} \longrightarrow HN_3 + Na^+ \qquad (3)$$

$$3HN_3 + NO_2^- + H^+ \longrightarrow 3N_2 + NO_2\uparrow + 2H_2O \qquad (4)$$

Titration is performed with thiosulfate, using starch solution as an indicator. If molecular iodine (I_2) is present, a blue color appears. Titration reduces molecular iodine to iodide ions (I^-) and the blue color disappears:

$$2Na_2^{++} + S_2O_3^{=} + I_2 \longrightarrow Na_2S_4O_6 + 2Na^+I^- \qquad (5)$$

Knowing the amount of thiosulfate used, we can determine the amount of I_2 and hence the amount of dissolved oxygen, since oxygen was used to produce iodine (reactions 1 and 2). Because 1 ml of 0.025N sodium thiosulfate titrant is equivalent to 0.2 mg/liter DO, when a volume equal to 200 ml of original sample is titrated, each milliliter of sodium thiosulfate titrant used is equal to 1 mg/liter DO.

QUANTITATIVE ANALYSIS OF DISSOLVED OXYGEN --WINKLER AZIDE TITRATION METHOD

Equipment:

Dissolved oxygen bottles or 250-300 ml Erlenmeyer flasks with stoppers

1500 ml flasks with stoppers

scale

source of heat

thermometers

10 ml, 50 ml, and 250 ml graduated cylinders

2 pipettes graduated in 2 ml units

50 ml burette graduated in 0.1 ml units

Reagents:

1. Reagent I - Manganese Sulfate Solution: Dissolve 480 g $MnSO_4 \cdot 4H_2O$, 400 g $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$ in distilled water. Filter and dilute to 1 liter.

2. Reagent II - Alkali-Iodide-Azide Reagent: Dissolve 500 g sodium hydroxide, NaOH, and 150 g potassium iodide, KI, in distilled water and dilute to 1 liter. To this solution, add 10 g sodium azide, NaN_3, dissolved in 40 ml of distilled water. This reagent should not give a color with starch solution when diluted and acidified.

3. Reagent III - Sulfuric Acid: Use concentrated reagent grade sulfuric acid, H_2SO_4. Handle with care!

4. Starch Solution: Prepare a paste of 5-6 g soluble starch in a small amount of distilled water. Pour this paste into 1 liter of boiling distilled water. Allow the paste and water to boil a few minutes and let settle overnight. Use the clear supernatant. This solution may be preserved with 1.25 g salicylic acid/liter or by the addition of a few drops of toluene.

5. Sodium Thiosulfate Stock Solution, 0.10 N: Dissolve 24.82 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 1 g NaOH/liter.

6. Working Sodium Thiosulfate Titrant, 0.025N: Dilute 250 ml sodium thiosulfate stock solution to 1 liter.

7. For standardizing the sodium thiosulfate with $K_2Cr_2O_7$ solution: Dissolve approximately 2 g KI, free from iodate, in an Erlenmeyer flask with 100 to 150 ml distilled water. Add 10 ml of 3.6N H_2SO_4 followed by exactly 20 ml of standard dichromate solution (reagent, step 8) and place in the dark for 5 minutes. Dilute to 400 ml and titrate with the 0.025N thiosulfate, adding starch toward the end of the titration when a pale straw color is reached. If the sodium thiosulfate is 0.025N, exactly 20 ml of this titrant will be required to reach the titration endpoint. If necessary, adjust the sodium thiosulfate to 0.025N by adding $Na_2S_2O_3 \cdot 5H_2O$ if the above titration required more than 20 ml. If the titration required less than 20 ml, add boiled and cooled distilled water. Again, check by titration.

8. Standard Potassium Dichromate Solution, 0.025N: Weigh out 1.226 g of $K_2Cr_2O_7$, previously dried in an oven at 103°C for 2 hours. Prepare the solution in a volumetric flask by diluting the $K_2Cr_2O_7$ to a liter. A solution of $K_2Cr_2O_7$ equivalent to 0.025N sodium thiosulfate contains 1.226 g/liter $K_2Cr_2O_7$.

Procedure for Measuring Dissolved Oxygen: Winkler-Azide Method

1. To your water sample, as collected in a dissolved oxygen bottle or in a 250-300 ml Erlenmeyer flask, add 2 ml of Reagent I followed by 2 ml of Reagent II by pipetting below the surface. Be careful not to stir up the sample. Stopper with care so that air bubbles will be excluded. Mix the contents by inverting the bottle 15 times. A brown precipitate should appear. The dissolved oxygen is now fixed (equation 1, p. 4). If collecting in the field, this step should be performed immediately after collection.

2. Allow the precipitate to settle, leaving about 100 ml of clear supernatant above the precipitate. Carefully remove the stopper and immediately add 2 ml of Reagent III. Let the acid run down the neck of the bottle.

Restopper and mix by inverting the bottle slowly until dissolution is complete. Note: At this point, if the solution is colorless, there is no dissolved oxygen in the sample.

3. Measure 203 ml of treated sample and pour it into a 250 ml graduated cylinder. Pour this solution into a 500 ml Erlenmeyer flask. Fill the burette with 0.025N sodium thiosulfate.

4. Titrate with the thiosulfate until the sample has a pale straw color. Add 1-2 ml of starch solution and continue the titration until the blue color disappears. This is the end point of titration.

5. Carefully read the amount of titrant needed from the burette. The amount required is exactly equivalent to the dissolved oxygen concentration in the water. If 7.5 ml of 0.025N thiosulfate is required, the dissolved oxygen concentration in the water is 7.5 mg/liter.

Experiments:

A. Dissolved Oxygen vs. Salinity

1. Prepare four salt solutions of 10,20, 40, and 100 g NaCl/liter by dissolving that number of grams of NaCl (uniodized) in one liter of water. Be sure there will be enough for each lab station. Each station will require 300 ml of each solution.

2. Fill labeled dissolved oxygen bottles or 250-300 ml Erlenmeyer flasks with these solutions.

3. Measure the DO content of each salt sample. Temperature should be the same in all samples.

4. Plot DO vs. salinity.

5. What can you conclude? Why?

6. How do your results compare with those of Table 1, p. 3, at 23°C? (Note: 1,000 mg/liter = 1.0 g/liter)

B. Dissolved Oxygen vs. Temperature

1. Adjust four water samples to different temperatures: 4°C (refrigerator), 23°C (room temperature), 35°C, and 60°C. Be sure there is enough of each sample for each station.

2. Fill labeled dissolved oxygen bottles or 250-300 ml Erlenmeyer flasks with these solutions.

3. Measure the dissolved oxygen of each temperature sample. Perform step 1 of the Winkler procedure (p. 6) as soon as possible and measure the temperature of the sample at that time.

4. Plot dissolved oxygen vs. temperature.

5. What can you conclude? Why?

6. How do your results compare with Table 1 at 0 mg/liter chloride?

C. Dissolved Oxygen vs. Organic Matter

1. Prepare samples of organic matter. If possible, use activated sludge from a waste treatment plant or decaying organic matter such as rotting leaves in a stream. Add samples to two flasks filled with tap water and let sit for a few days. One flask should be left open; the other should be capped. Also, use a control of plain tap water. Be sure there is enough of each sample for each lab station.

2. Fill labeled dissolved oxygen bottles or 250-300 ml Erlenmeyer flasks with these samples.

3. Measure the dissolved oxygen.

4. What can you conclude about the effect of organic pollution on dissolved oxygen?

EXERCISES:

A. Dissolved Oxygen and Types of Animals Found in the Water Environment

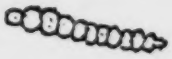
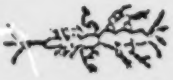
To more fully understand the effect of dissolved oxygen, look at the data from a study published in the Ohio Journal of Science (see Table 2, p. 9). The bottom of western Lake Erie was surveyed for organisms in the same manner several different times: once in 1929, once in 1930, and again in 1958. A measured area was set off and all material in this area to a depth of one inch was removed. The scientists counted and classified all organisms. Study the chart on the following page carefully.

1. Make a bar graph of the data, using different shadings to indicate each year.

2. If you were the scientist analyzing this data, what would you conclude about the biological history of western Lake Erie and the factors which influenced it?

TABLE 2

Bottom-dwelling Organisms in Western Lake Erie*

Kind of organism		Requires high oxygen content in water	Animals/sq. meter of bottom		
			1929	1930	1958
leeches		no	6	4	37
small worms		no	6	3	559
mayfly larvae		yes	312	515	49
midge fly larvae		no	81	22	257
snails		yes	12	24	7
fingernail clams		no	16	8	55
caddis fly larvae		yes	20	1	3

* Life Science Investigations: Man and the Environment, p. 277, © 1971 by Educational Research Council of America. Reprinted by permission of Houghton Mifflin Company.

B. Water Quality in the Delaware River

Table 3 below presents the average DO concentration for several of the sampling stations (see Fig. 1, p. 11) for four three-month periods in the year 1964.

1. Plot the DO concentrations vs. station number. Use a different color for each of the four time periods.

2. At any one station, what can you conclude about the seasonal variation of dissolved oxygen?

3. What can you conclude about the distribution of dissolved oxygen in this region? What factors may cause this distribution?

4. What climatic and physical factors may cause this distribution?

TABLE 3

DISSOLVED OXYGEN DATA FOR 1964

Station Name & Approximate Location	Average Dissolved Oxygen (mg/liter) Jan-Mar	Apr-June	July-Sept	Oct-Dec
Fieldsboro, NJ Section 2	12.2	9.6	6.7	9.5
Burlington, NJ Section 4	12.0	8.3	5.2	8.2
Torresdale, PA Section 6	12.1	8.3	6.4	7.8
Tacony-Palmyra Br. Section 8	11.9	7.7	4.7	6.6
Ben Franklin Br. Section 12	10.5	5.5	1.2	2.9
Phila. Navy Yard Section 15	9.7	5.2	0.7	1.6
Eddystone, PA Section 17	8.7	5.1	1.0	1.8
Marcus Hook, PA Section 19	9.0	3.4	1.7	3.2
Del. Mem. Br. Section 22	9.8	4.6	4.5	7.4
Reedy Island, DE Section 29	11.8	7.7	7.2	9.8

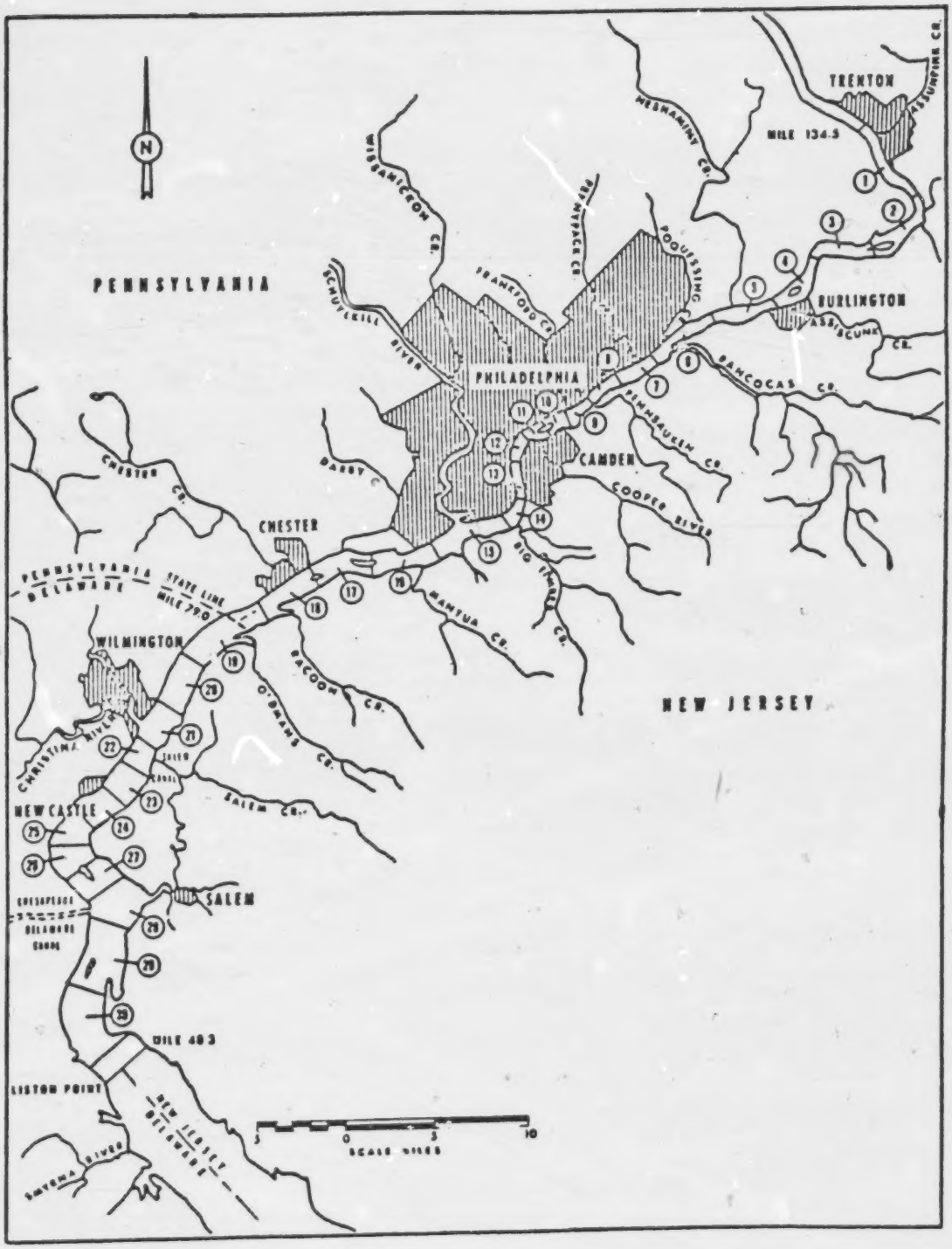
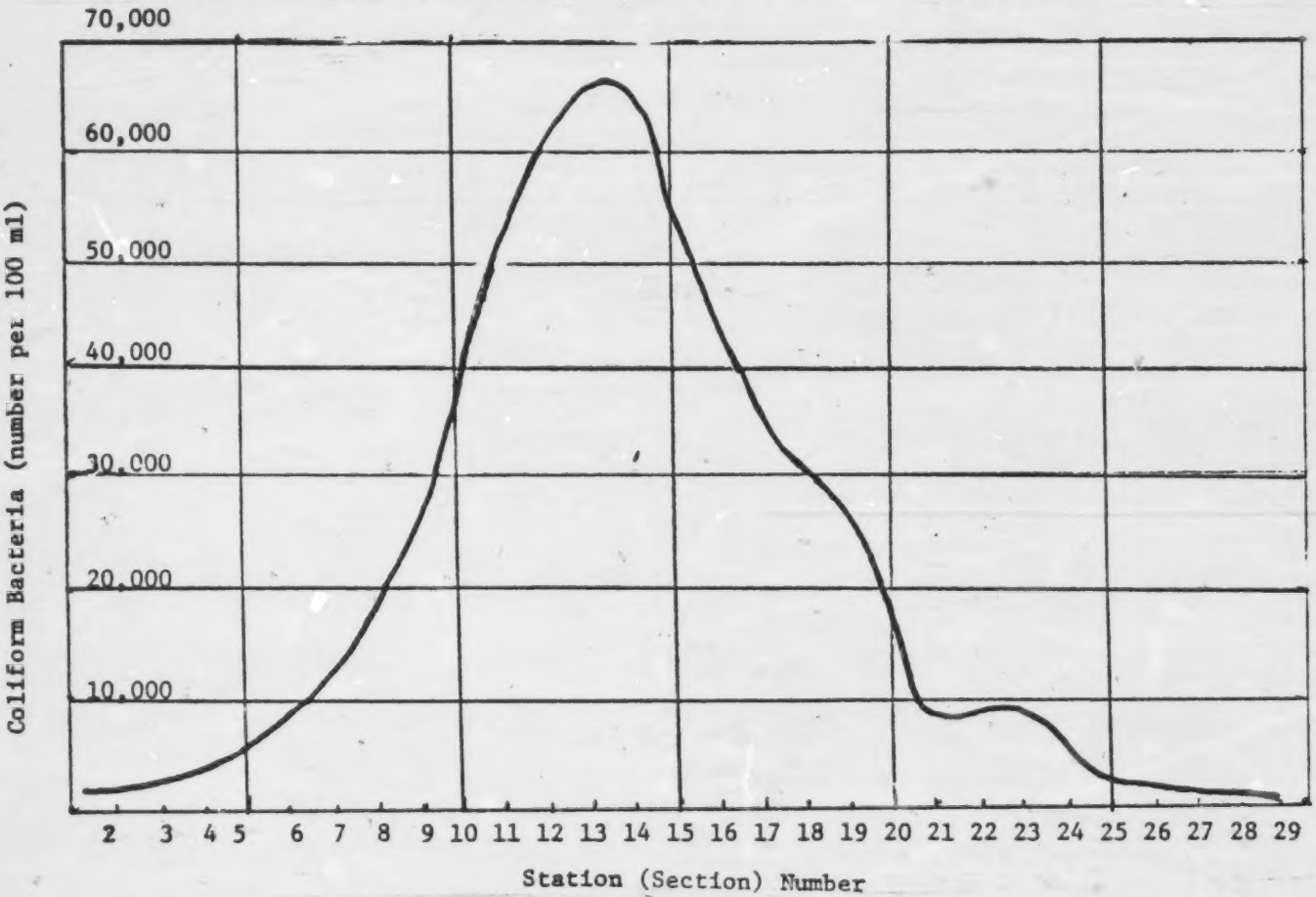


Fig. 1. - Map of Delaware Estuary showing section breakdown.

Coliform bacteria are normally found in the intestines of humans and other mammals and aid in digestion. Quantities of these bacteria are excreted in the feces and are found in raw sewage. If untreated sewage is allowed to enter rivers (either by dumping or seepage), coliform bacteria will be found there. Coliform bacteria can be found in the Delaware River, as illustrated in Figure 2, below.

Figure 2: Profile of mean summer coliform bacteria along the Delaware River



5. What does the presence of coliform bacteria in a body of water indicate?

6. What may lead to the high levels of coliform bacteria between Stations 9 and 17?

7. Compare your graph of seasonal DO in the Delaware River and the coliform bacteria graph on this page. Does there appear to be a correlation between the two?

8. From the laboratory results and this data, what would you conclude is the cause of the low DO levels?

9. If you were an environmental marine biologist asked to submit a report to the Environmental Protection Agency, what would you conclude about the types of aquatic life existing in the Delaware River Estuary from Trenton, NJ, to Reedy Point, Delaware? What evidence would support your conclusions?

C. Additional Exercises

1. Collect water samples from various types of water in your area, such as a fast-flowing, well-aerated stream, a normally flowing stream, a lake or pond, a stagnant pond, a bay or an area near a sewage treatment plant effluent. You may also want to sample these areas at different times of the year.

2. Collect these samples directly into the dissolved oxygen bottles or the 250-300 ml Erlenmeyer flasks. Be careful not to agitate the samples. Immediately perform step 1 of the Winkler method to fix the oxygen. Note the temperature and label the sample as to its site. You may wish to make a field kit containing several collecting bottles, a bottle rack, Reagents I and II, two 2 ml graduated pipettes, a thermometer, labels, and pencils.

3. In the lab, before analyzing for DO, try to predict the amount of DO contained in the samples based on the nature of the collecting site, temperature, and salinity.

4. Measure the DO. How close are your results to your predictions?

5. Record your findings and the reasons for the DO levels you found.

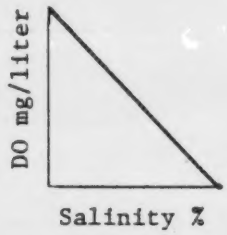
6. No animal can live where there is no oxygen, but different kinds of aquatic life require different levels of dissolved oxygen. For example, a trout requires 6 ppm DO while a perch requires only 2 ppm DO. Would both fish be able to survive in your water sample? Look up information on oxygen requirements of different kinds of aquatic life that might be found in your area and see if they could survive in your samples.

SUGGESTED ANSWERS

Experiments, pp. 7-8

A. Dissolved Oxygen vs. Salinity

4.

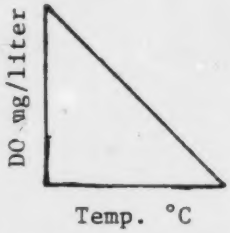


5. The greater the salinity of the water, the lower the DO content. The presence of salt in water reduces the capability of the water to retain oxygen.

6. Your observations.

B. Dissolved Oxygen vs. Temperature

4.



5. The higher the temperature of the water, the lower the DO content. Higher temperatures also reduce the capability of the water to retain oxygen.

6. Your observations.

C. Dissolved Oxygen vs. Organic Matter

4. When organic matter is present, bacteria start the decay process, which uses oxygen. This depletes the dissolved oxygen. The uncapped flask should have a higher DO content since oxygen is generally able to enter the water from the atmosphere.

SUGGESTED ANSWERS (cont.)

Exercises, pp. 8-13

A. Dissolved Oxygen and Types of Animals Found in the Water Environment

1. See p. 17 for a sample graph.

2. The water quality in Lake Erie has decreased, as evidenced by an increase in the number of organisms present which are able to survive in water with a low dissolved oxygen content and a decrease in the number of organisms present which need a high dissolved oxygen content. Because the disappearance of organisms which require a high dissolved oxygen content parallels a time when industry, municipal development and their resulting pollution have boomed, it would seem that pollution may be the cause.

B. Water Quality in the Delaware River

1. See p. 18 for graph.

2. As illustrated by the low DO levels during July-Sept., the onset of warmer weather brings a decrease in the dissolved oxygen content. This is due to the reduced ability of warm water to retain oxygen and increased bacterial activity as water temperatures rise.

3. The dissolved oxygen content decreases greatly near the city of Philadelphia and the petroleum refinery area of Marcus Hook. The dumping or seepage of large amounts of municipal and industrial organic wastes in this area may cause this decrease.

4. Wind can influence the dissolved oxygen content by causing waves and aeration of the surface waters. Tidal action can increase dissolved oxygen in a bay or river by bringing in large amounts of well-oxygenated ocean water. Air temperature affects water temperature, which as shown before, affects the capability of water to retain dissolved oxygen.

5. The presence of organic pollution such as raw sewage.

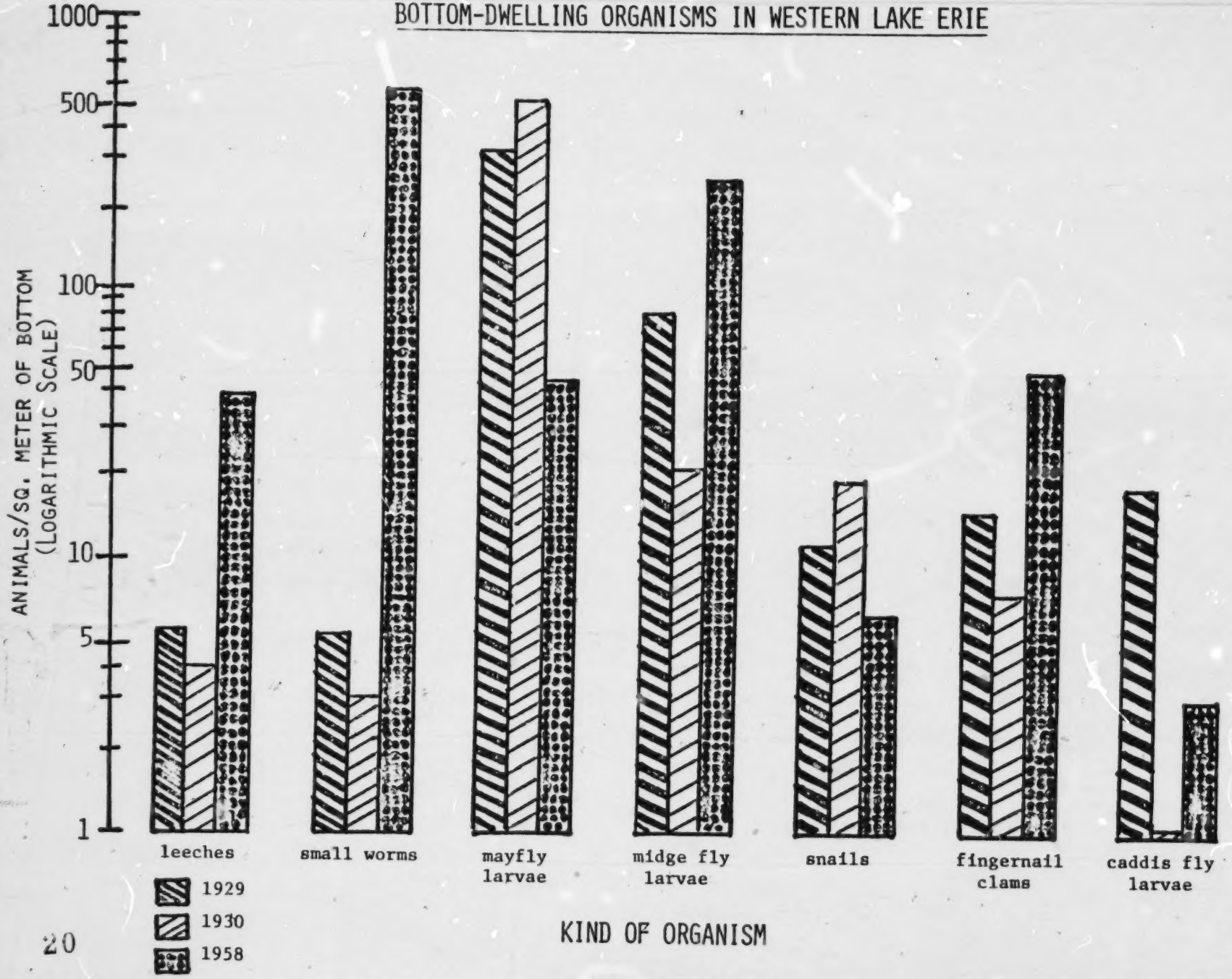
6. Municipal and industrial wastes being dumped from Philadelphia.

7. Yes. A high coliform bacteria count correlates with a low dissolved oxygen content.

8. Three factors may be at work. The high bacterial and organic content of municipal and industrial wastes leads to a depletion of dissolved oxygen through bacterial decay of organic matter. Increased summer water temperatures not only result in decreased water oxygen solubility but also increase the activity of bacteria. Another factor that may affect the dissolved oxygen content, although to a lesser extent in this area, is salinity. In the summer, the freshwater flow from upstream is not as great and the salinity of the river increases, resulting in a further reduction of the water's ability to retain oxygen.

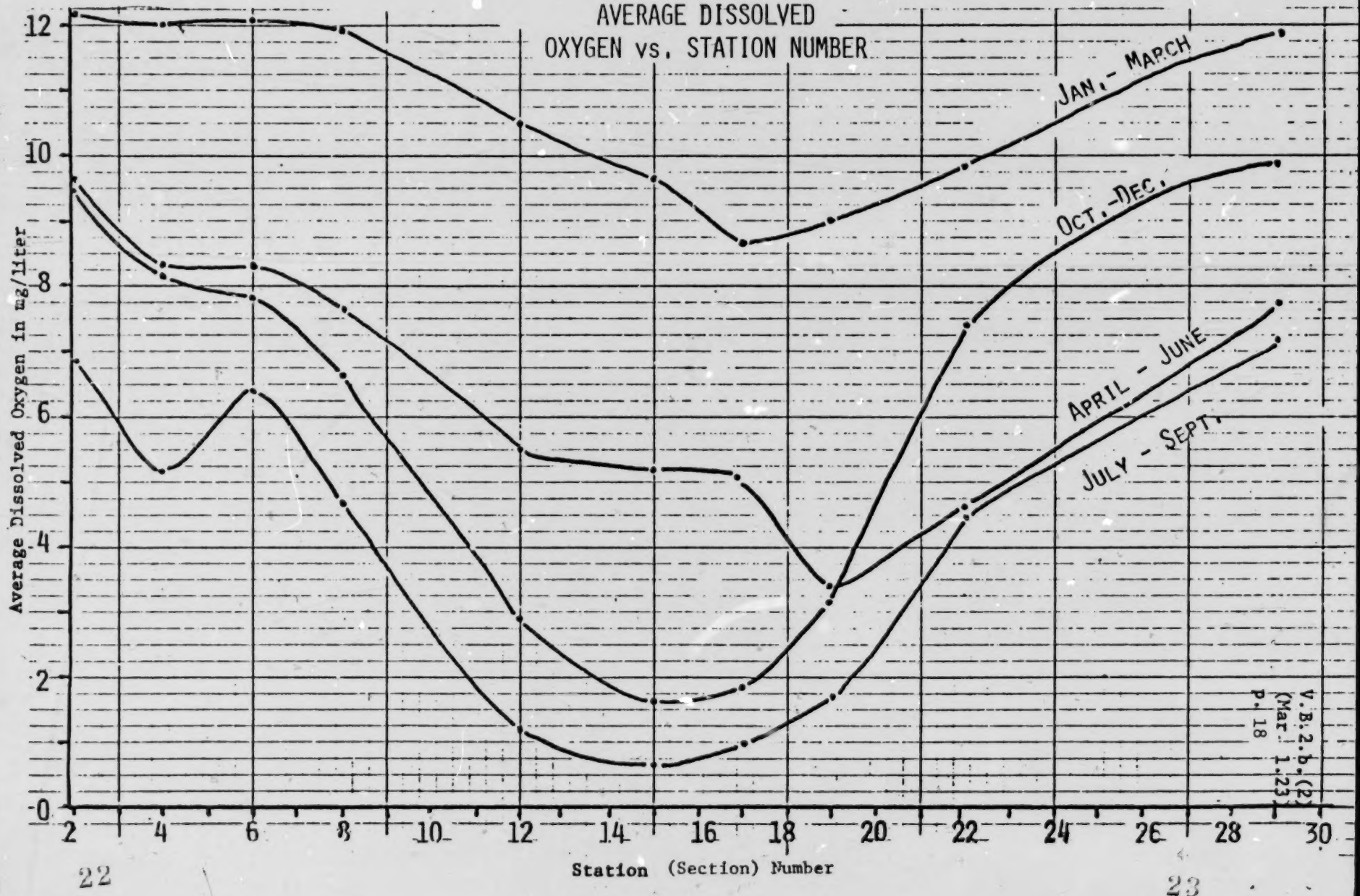
9. As a result of the data above, one would conclude that only those few species capable of tolerating low salinities and high pollution would be present in the section of the Delaware River from Philadelphia to Marcus Hook. The remainder of the study area appears to have good water quality so that a normal abundance of plant and animal life would probably be found.

BOTTOM-DWELLING ORGANISMS IN WESTERN LAKE ERIE



V.B.2.b.(2)
(Mar. 1,23)
p. 17

Plot of Data from Table 3, p. 10



V.B.2.b.(2)
(Mar. 1.23)
P. 18

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No. 1 Nature of the Crisis
No. 4 Freshwater Pollution
No. 5 Marine Pollution
No. 6 Pollution Control

Film: "Crisis in the Estuary," Instructional Resources Center, University of Delaware, Newark, DE 19711.

Other learning experiences relevant to this topic include:

V.B.(Mar. 4.2) "Teacher Resource Guide to the Film Crisis in the Estuary."
V.B.1.c.(Mar. 4.22) "Testing Water for Bacterial Pollution."
V.B.2.b.(2)(Mar. 1.23) "Dissolved Oxygen Measured Qualitatively."
I.B.3.(Mar. 3.33) "Collecting and Cultivating Marine Bacteria."
V.B.1.c.(1)(i) "Water Quality and Treatment."
V.B.2.b.(2) "Nitrate Water Activities."
I.B.3. "A Study of Pond and Stream Life near Newark, Delaware."

The learning experiences are available from Population-Environment Curriculum Study, 310 Willard Hall, University of Delaware, Newark, DE 19711.

COLLECTING AND CULTIVATING MARINE BACTERIA

A Learning Experience for
Coastal and Oceanic
Awareness Studies

Produced by

MARINE ENVIRONMENT CURRICULUM STUDY
MARINE ADVISORY SERVICE
UNIVERSITY OF DELAWARE

and

POPULATION-ENVIRONMENT CURRICULUM STUDY
COLLEGE OF EDUCATION
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DEPARTMENT OF PUBLIC INSTRUCTION

DEL MOD SYSTEM

DUPONT EDUCATIONAL AID COMMITTEE

TITLE: COLLECTING AND CULTIVATING MARINE BACTERIA

*CONCEPT: I.B.3.

I. The earth is a finite natural system.

B. All living things have arisen from and are dependent upon the natural system of the earth.

3. MOST ORGANISMS INTERACT IN BALANCE WITH THEIR PHYSICAL AND BIOTIC ENVIRONMENT.

**MARINE CONCEPT: 3.33

3. Marine organisms interact in complex ecosystems.

3.3 Marine ecosystems depend on a constant flow of energy and the recycling of materials.

3.33 MARINE BACTERIA REDUCE ORGANIC MATERIALS TO SIMPLER FORMS.

GRADE LEVEL: 10-12

SUBJECT: Biology

CLASS PERIODS: Various

AUTHOR: Williams

* From A Conceptual Scheme for Population-Environment Studies, 1973. Cost $2.50.

** From Marine Environment Proposed Conceptual Scheme, 1973. No charge.

Both conceptual schemes are available from Robert W. Stegner, Population-Environment Curriculum Study, 310 Willard Hall, University of Delaware, Newark, DE 19711.

INTRODUCTION

It is now recognized that bacteria are widely distributed in the sea. The influence of marine bacteria on chemical and biological phenomena in the sea is of major importance. They liberate nutrients for plants by decomposing marine plant and animal residues. They contribute to the regeneration of phosphate, transform sulphur compounds, oxidize ammonia to nitrate, and in various ways affect the chemical composition of seawater and bottom deposits. The bacteria themselves are an important food source for many kinds of small marine animals. The distribution of oxygen in the sea is to a considerable extent influenced by their enormous consumption of this gas, probably equal to or greater than that of all other organisms combined.

As mentioned before, the overall result of bacterial activity is to break down the accumulations of organic matter from the remains of plants and animals. This then releases their chemical constituents in the form of simple soluble inorganic ions and gases that can be utilized again by the plants. This process is termed the "mineralization of organic matter." It is of the utmost importance in the cycling of available chemicals necessary for life and thus for the continuation of life on earth. (See Appendix , p. 9)

Marine bacteria are found everywhere in the oceans and estuaries, but their frequencies vary with certain conditions. The largest populations ordinarily occur in water along or near the shore if the water is rich in organic material. The greatest numbers of bacteria in this area are found at the mud-water interface. Thus, shore waters may contain or support 50,000 to 400,000 bacteria per milliliter. The large numbers in coastal waters are usually related to nutrients introduced by comparatively heavy runoff from the land; along arid coasts the populations differ little from near shore to the open sea.

Marine bacteria have several notable physical characteristics. They are generally smaller than non-marine forms. A large portion (about 95 per cent) are Gram-negative rods, and most of these are motile, flagellated forms. Cocci are infrequently found. Many marine bacteria are sedentary and attach themselves to the substrate with polymeric fibrils called pili, mucilaginous slime, and occasionally with holdfasts. It is possible that they find more nutrients adsorbed on solid surfaces than in free solution.

A striking characteristic of most marine bacteria that have been cultured is their color. Nearly 70 percent are pigment producers. Over 30 percent are yellow or orange; brown pink and green pigments occur less frequently. A considerable number show flourescence, usually of a greenish hue.

Marine bacteria have a wide range of temperature tolerance, but are much more sensitive to high temperatures than most fresh-water or terrestrial forms. The optimum temperatures for a large proportion of marine bacteria thus far cultured, range between 18 and 22 degrees centigrade, but most remain active and may even grow slowly at 0 to 4 degrees C. A high proportion of bacteria from seawater or marine muds are facultative anaerobes, but most of them apparently lose their ability to grow anaerobically after prolonged culture under aerobic conditions.

Terrestrial species of bacteria commonly occur in inshore marine waters which they reach by runoff from the land and by precipitation from continental air masses. Some of these species can become adapted to seawater conditions, but by and large the terrestrial forms cannot compete with marine bacteria in seawater and do not survive very long.

Marine bacteria play several other important roles besides their role in the mineralization of organic matter. Many are ingested along with minute detrital particles by filter feeders, such as bivalve mollusks and copepods. Marine bacteria are also eaten by small animals such as protozoans, rotifers, and nematodes. In localized areas where the organic content of the water is above average, and the weather is very calm, marine bacteria sometimes deplete the oxygen in the surface waters, causing the death of many animals. Not only does the decreased oxygen cause the death of bottom organisms, but the lack of oxygen causes very significant geological changes to occur in the sediment.

The problems of collecting bacteria samples at sea, particularly water samples, have led to the use of more than a hundred different types of devices, most of which are suitable only for collecting at the surface, or in shallow water. Collection of mud samples presents fewer problems because the bottom samples always contain hundreds of thousands of times as many bacteria as the overlying water does. Collection of mud samples also minimizes many contamination problems common to water sampling. Samples of bottom deposits may be collected with standard geological coring tubes, some of which may collect cores of five or more meters in length.

Some kinds of marine bacteria may be collected by the simple and inexpensive method of submerging ordinary glass microscope slides. These may be examined by phase-contrast microscopy or by ordinary microscopy after direct staining. Special fixation methods are not always necessary. Bacteria from such slide collections may also be isolated by plating on nutrient agar.

Various kinds of nutrient media have been used for isolating marine bacteria. Ordinarily, these are prepared with seawater because most forms grow best in seawater media. Most successfully culture less than one percent of the bacteria present in a sample. (See Appendix) Some investigators have reported that after bacteria are cultured in seawater media for several weeks or months, a considerable number of these marine bacteria develop an ability to grow in freshwater media. Generally, however, the bacteria are fastidious in their salt requirements and tolerate neither increases nor decreases.

Marine bacteria are directly important to man in causing spoilage of marine food products and deterioration of nets, ropes, and cables and in initiating the fouling of all submerged surfaces such as boat bottoms.

In summary, marine bacteria play a major role in influencing the physical, chemical, biological, and geological processes in the oceans and estuaries.

Laboratory Investigation

Materials and Equipment:

autoclave (or large home pressure cooker)
distilled water
cotton
disinfectant
incubator
pyrex beakers and flasks
nutrient agar
glass stirring rods
aluminum foil
thermometer(s)
peptone
aged and filtered sea water from collecting site for agar medium (adjust pH to correspond to sample collected)
glass-stoppered bottle
Erlenmeyer flask
inoculating loop
crystal violet stain, 2% aqueous
Bunsen burner or alcohol lamp
cheesecloth

Procedure:

I. Collect a seawater sample for bacteriological study.

A. Prepare a sterile sampling bottle.
1. Wash, rinse in distilled water and air dry an 8 oz. glass-stoppered bottle.
2. Make a cotton plug by wrapping a piece of cotton in a small piece of cheesecloth. Insert the plug into the bottle mouth. Loosely wrap the bottle stopper with foil.
3. Autoclave the bottle for 25 minutes at 15 psi.
4. After removing the bottle from autoclave, seal the foil.

B. Collect the seawater sample.
1. Hold the bottle under the water and remove cotton plug.
2. When the bottle is full, remove foil from stopper and insert stopper into bottle.

II. Cultivate seawater bacteria.

A. Prepare sterile petri dishes.
1. Wash, rinse in distilled water and dry 3 to 5 petri dishes.
2. Place the cleaned dishes in a clean storage area or on the autoclave tray.

B. Prepare agar medium for petri dishes (this may be done by one group of students for the whole class).
1. Estimate agar water requirements for the class on the basis of 50 ml of agar per plate. Use the following formula for the marine bacteria medium:
- 1 liter of aged, filtered, pH adjusted seawater
- 10 grams of peptone
- 15 grams of agar
- (There are additional formulas for this medium; the students might seek out other references on this formula.)
2. Put seawater in a Pyrex beaker and heat to a slow simmer on a hot plate or stove.
3. Add the peptone and agar. Stir constantly.
4. Heat to mixture to 100° C. When the agar has dissolved, remove beaker from the heat source.
5. Pour about 125 ml of the (hot) liquid medium into a 250 ml flask. Each student group should have one flask with about 125 ml of the agar medium.
6. Plug each flask with a twisted wad of cotton.

C. Sterilize the petri dishes and agar medium.

1. Pour 1 liter of distilled water into sterilizer. Replace bottom basket and grid.
2. Place sterilizer on stove or hot plate.
3. Place petri dishes and agar flasks in basket.
4. Put on the sterilizer cover and tighten all bolts.
5. Open the air release valve.
6. Turn on the heat source.
7. When steam blows strongly through the release valve for about one minute, close the valve.

8. Monitor the pressure guage at all times.
9. When the guage reads 10 psi, begin turning down the heat.
10. When the pressure reaches 15 psi, adjust the heat source so that the pressure does not exceed 16 psi and does not fall below 15 psi.
11. Maintain the pressure for 25 minutes, then turn off the heat.
12. Allow the sterilizer to cool to 0 psi.
13. Open the air release valve.
14. Unlock the cover of the sterilizer and remove it.

D. Prepare sterile petri dishes for cultivation.
1. Wash hands and desk-top work area with disinfectant.
2. Place petri dishes on cleaned desk.
3. Allow the dishes to cool and dry.
4. Set up a propane or bunsen burner.
5. When the agar flask(s) has cooled so it can be safely handled without discomfort you are ready to pour out the liquid medium.
6. Light the burner.
7. Remove cotton plug, then flame the opening of the flask. Lift one side of the petri dish lid far enough to allow you to pour 2-3 mm of agar into the bottom of the petri dish. Do this quickly. Flame the flask mouth before and after each pouring. Replace the cotton plug when finished.
NOTE: When the plates have cooled and the agar has solidified, proceed with the next step.

E. Streak the medium with the seawater sample
1. Light the burner.
2. Flame the bacteriological inoculation loop.
3. Remove the stopper from the sample bottle.
4. Flame the mouth of the bottle.
5. Slowly insert the wire loop and withdraw a loopful of the sample. Be careful not to touch the sides of the bottle. Flame the bottle mouth and replace the stopper.
6. Streak the plate, being careful not to break the surface of the agar medium. Again, one side of the petri dish lid should be raised only as far as needed to allow you to streak the agar. (Check with your teacher for the proper technique.)
7. Flame the wire loop and bottle mouth, then repeat the process for one more plate.
8. The third plate is to be used as your control and should not be opened.

F. Incubate the plates.
1. Adjust the incubator temperature to 27° C. Place a dish of distilled water in the incubator for additional moisture.
2. Invert your marked (with your name) dishes and place them into the incubator. Let them remain there for the next three or four days.

III. Determine the types of bacterial growths.

A. Make daily observations of the following data:
1. Number of colonies present
2. Color, shape and size of each colony

B. Make a smear of each type of bacterial growth.
1. With sufficient growth of bacteria colonies after three of four days in incubation, use the following procedure:
2. Place a drop of sterile seawater on a clean slide.
3. Flame the inoculating loop.
4. Pick off a small amount of a colony with the loop and mix it into the drop of water on the slide. Allow this smear to dry.
5. Pass the smear through a flame three times to fix the bacteria to the slide.
6. Gently flood the smear with crystal violet for 30 seconds.
7. Rinse the slide in a beaker of distilled water and gently blot dry.
8. Repeat this procedure for as many different kinds of colonies that may be growing on the agar medium. (Never leave the dish uncovered after removing a piece of the bacterial growth.)

C. Record microscopic observations.

1. Place a drop of immersion oil on the stained slide and carefully focus under the oil immersion lens.
2. Make a rough sketch of each type of bacterial cell you observe.
3. Repeat this procedure for each prepared slide or smear that you or your group have done.

IV. Submit laboratory data.

A. Label each slide with your name and a number. Store these in a safe place.

B. Complete the data sheet on p. 10.

SUMMARY

The marine environment has many microorganisms that are different from those found in fresh waters. One difference is that in terrestrial and freshwater environments, the higher plants are far more important in the biomass than the lower plants; in the marine environment, the reverse is true. Apart from the seaweeds around the coasts, the major part of the plant life in the water is microbial. The significance of marine bacteria in the study of the oceans lies in their numbers and biomass and in their role in a number of chemical and physical processes. In estuaries and intertidal zones, the bacteria are very important, even if only for their rapid rate of turnover of nutrients.

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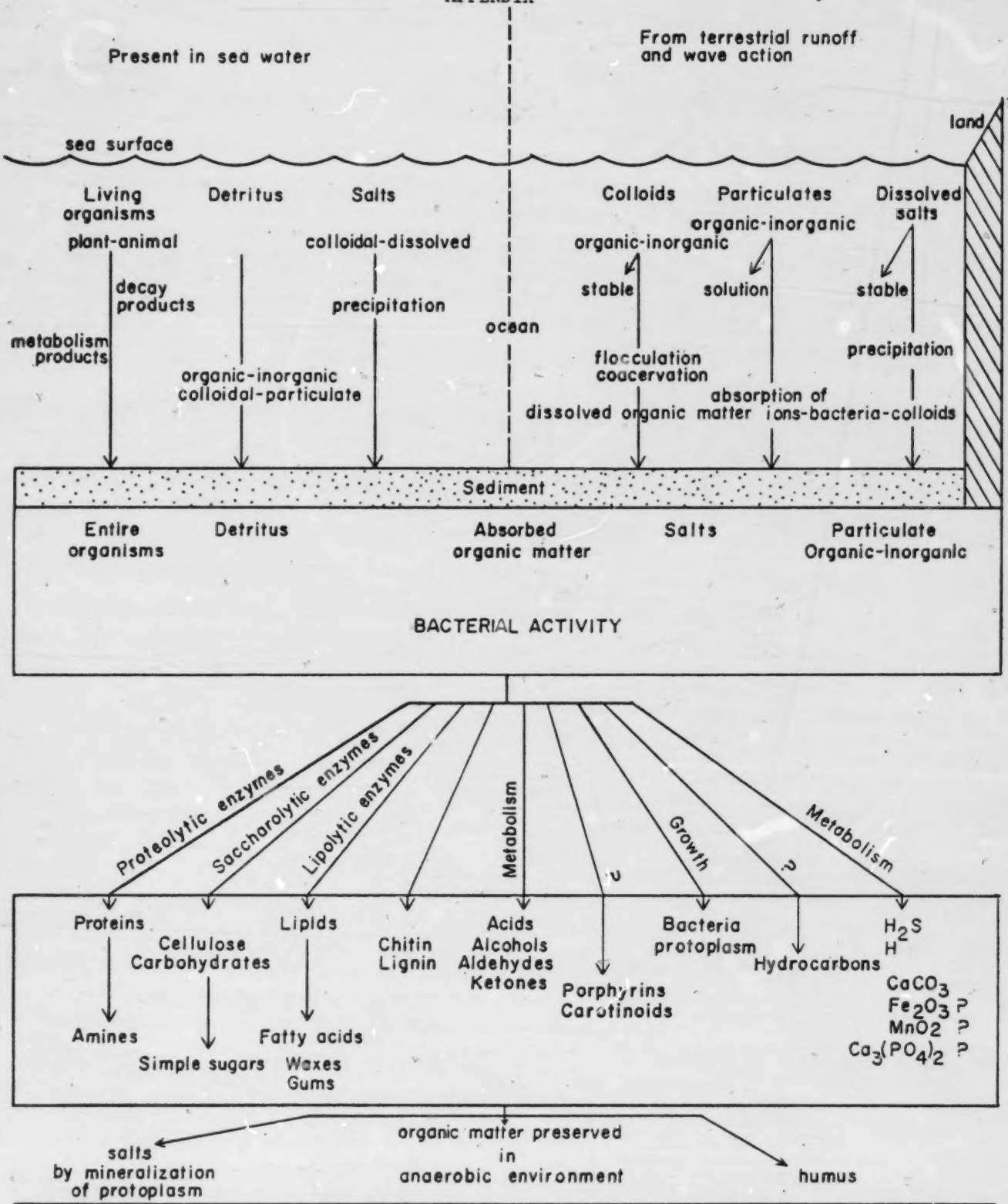
APPENDIX



FORMATION OF MARINE SEDIMENTS AND THE BACTERIAL ACTIVITY UPON THE VARIOUS SUBSTRATES (Redrawn from McGraw-Hill Yearbook of Science and Technology, 1963)

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APPENDIX . . .

Various kinds of nutrient media . . . . . (from page #4)

The following formula allows the cultivation of many marine aerobic heterotrophs:

Bacto-peptone - 5.0 grams
Ferric phosphate - 0.1 grams
Bacto-agar - 15 grams
Aged seawater - 1000 grams

* * * * * *

DATA SHEET NAME _____

Location of collecting site:

_____

Date of Collection Description of Site

_____

Water Depth _____

Distance from Shore _____

Water Temperature _____

pH of Sample _____

Cellular Characteristics for each smear

_____

_____

Control Results _____

Type of Stain Used _____

Comments / Remarks _____

_____

* * * * * *

TO RECOGNIZE, RECORD, AND ANALYZE CHARACTERISTICS OF A SANDY BEACH ENVIRONMENT

A Learning Experience for
Coastal and Oceanic
Awareness Studies

Produced by

MARINE ENVIRONMENT CURRICULUM STUDY
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CHRISTIANA FOUNDATION

DEPARTMENT OF PUBLIC INSTRUCTION

DEL MOD SYSTEM

DUPONT EDUCATIONAL AID COMMITTEE

TITLE: TO RECOGNIZE, RECORD AND ANALYZE CHARACTERISTICS OF A SANDY BEACH ENVIRONMENT

* CONCEPT: I.B.3.b.(1)

I. The earth is a finite natural system.

B. All living things have arisen from and are dependent upon the natural system of the earth.

3. Most organisms interact in balance with their physical and biotic environment.

b. Ecosystems are dynamic and subject to change.

(1) NATURAL POPULATIONS FLUCTUATE IN RESPONSE TO ENVIRONMENTAL CONDITIONS.

** MARINE CONCEPT: 2.4

2. The oceans interact with the earth and its atmosphere.

2.4 MATERIALS CARRIED FROM LAND MASSES INFLUENCE THE PHYSICAL AND CHEMICAL FEATURES OF COASTAL ZONES.

GRADE LEVEL: 10-12

SUBJECTS: Science, Biology

CLASS PERIODS: Various

AUTHOR: Williams

* From A Conceptual Scheme for Population-Environment Studies, 1973. Cost $2.50.

** From Marine Environment Proposed Conceptual Scheme, 1973. No charge.

Both conceptual schemes are available from Robert W. Stegner, Population-Environment Curriculum Study, 310 Willard Hall, University of Delaware, Newark, DE 19711.

INTRODUCTION

It is the intention of the following learning activity to acquaint the student with several aspects of a coastal region, specifically the sandy beach and its environment. This self-contained study is designed to be a primary guide to the student. The activities described can be done by the student as they are written or used as a guide for the students to develop their own activity. The teacher is to act as a resource and reference aid.

There will be a minimum of prerequisite reading material. Reference will be made as to where the student may find material that deals with the various activities. It is highly recommended that the student locate these references and read them before approaching the teacher. Questions have been written into the activity solely as a guide for the student.

The 35 mm color slides accompanying this activity are intended to give the student a pictorial view of the beach and dune environment to be studied. They may be used at any point in the unit.

The learning activity presented here is intended for senior high school classes. The activity takes into account that the student has had some background in ecological principles, terminology, and how to use these principles in a practical way, whether in the classroom or in the field.

It is a wise practice to visit the field site personally beforehand and provide maps, charts, and tide tables for the students who are going to study a particular dune area. Also suggest proper clothing to wear and if they need to bring their own food.

Throughout the learning activity it might be helpful to encourage your students to modify or change the measurement activities. Feel free to let them use these methods to develop their own measurements. The Platt and Griffiths text is an excellent reference on measurement activities. Tait's book is another good source of field and lab exercises (along with Smith's text), pp. 253-264.

Materials Needed:

Metric rulers
Line or string (lots of it)
Plastic bags and buckets
5' wooden poles (2)
Identification books on plants
Thermometers
Shovels and trowels
Drying oven and dishes
Camera and film (students should take their own also)
Scales, triple beam or Metler balance
Graph paper (cm)
Colored pens and pencils, small rulers (with both scales)
Permission slips from parents if needed
Old clothes for field trips, insect spray and a band-aid or two; don't forget to invite the principal or a parent along for moral support - have fun!!

DUNE FORMATION AND TYPES OF DUNES

The principal feature of the dune environment is the sand that forms the dunes. From it stems nearly all the other characteristics that make up the peculiarities of the habitat.

> "There are two major phenomena that enable maritime dune formation to proceed. The first and most important is a supply of sand together with wind to move it, and the second is plant colonization. The supply of sand comes from the sand flats that are exposed at low tide, and on a windy day the movement of sand cannot only be seen but felt. Blown sand is deposited wherever the wind drops, as in passing over a hill or if it meets an obstacle, and the sand accumulates on the protected lee side. Plant colonization in the early stages assists in the further growth of dunes because plants represent obstacles, while the roots at the same time help to "fix" the sand already there, although this function is not important. As the cover becomes more and more complete and other dunes form in front and cut off the sand supply, so the dune gradually ceases to grow higher and is finally stabilized.
>
> "After dunes have been colonized and covered by plants, erosion may take place should the vegetation cover become broken. When this happens, the wind can act on the exposed bare sand and secondary movement of sand then takes place. In the early stages the evacuated area is known as a "blow-out", and, if not stopped, erosion proceeds until the whole dune has been removed, very often with the formation of a mobile dune." (Chapman, 1964, p. 133)

Dune formation often begins on the drift-line of a beach. Therefore, we may begin our study of sand dunes by a brief look at the drift-line vegetation.

> "Over a year more than one drift-line can be observed on a beach, but plants are generally restricted to the uppermost line, where there is the greatest stability." (Chapman, 1964, p. 134) "The foreshore drift-line which can be subject to storm tides, is poor in species, though these can be, and often are, the forerunners of dunes. The plants are generally scattered, and there is no development into any form of closed vegetation. Most of the species are annuals, and it is noteworthy that they belong to about three families (Cruciferae, Chenopodiaceae and Polygonaceae).
>
> "Sometimes the plants of the beach drift-line do not appear to be located in relation to the current drift-line, but if they are excavated it will be found that their roots are associated with a buried former drift-line. Although the species grow at the upper tidal

limit, they cannot endure prolonged immersion in sea water. Characteristically they have fleshy leaves and provision for water storage, either in the leaves...or in special hairs...such water storage being regarded as one means of meeting the considerable osmotic pressures of tissues that result from the roots being in highly saline water." (Chapman, 1964, p. 134)

Below is listed a classification of dunes:

"(a) Accumulation forms. In these dunes the vegetation becomes predominant and a successive series of new dunes arise in front of each other.

"(b) Fixation forms. These refer to the older dunes in a series of ridges, which have been gradually deprived of their sand supply. The valleys or hollows that exist between such dune ridges are known as 'slacks' or 'lows'.

"(c) Remanié forms. These result from erosion of the fixation forms as a result of wind attack if the vegetation cover is broken by some agency. If a dune undergoing erosion is stabilized, it represents a moderate remanié form, but in other cases practically the entire dune may be removed and in its place a mobile dune appears.

"(d) Parabolic dunes. These dunes can arise as above, or they may develop where the sand supply is particularly plentiful and the vegetation is inadequate, particularly in the center of the dune as compared with the sides. In such cases the higher central part continues to grow and move forward. The continual moving of the sand over the top forms a sharp lee side, and gradually a parabolic dune is formed, i.e., a dune concave to the direction of the prevailing wind, the sides of which are held back by the vegetation covering." (Chapman, 1964, p. 137)

Questions

1. Formation of dunes is due to what phenomena?
2. What causes a dune to become stabilized?
3. Where does the formation of a dune begin?
4. Name two characteristics of the "drift-line" plants?
5. Describe how the remanié form of dune is formed.
6. Why do you suppose the "drift-line" plants are annuals?

PRE-TEST

1. Discuss the differences between abiotic and biotic factors within the dune environment.

2. The following terms should be familiar to the student: community, ecology, zonation, limiting factor, habitat, and the units of the metric system.

3. Is temperature an abiotic or biotic environmental factor?

4. What kind of plants would you expect to find on a beach?

5. Do you consider the sandy beach an environment where both plants and animals can easily live and thrive? Explain.

6. Where do you suppose all that sand comes from that one finds on the beach and dunes?

7. Of the following terms, which one is not considered a good agent in affecting the presence of plants on a dune?

   (a) temperature
   (b) rainfall
   (c) wind
   (d) sunlight
   (e) all of these
   (f) none of these

8. Which description best fits a sandy beach?

   (a) hot and humid
   (b) steep and rocky
   (c) fine grain particles with a gentle slope
   (d) a place to go surfing

9. If you started walking back from the beach, what would you expect to find first?

   (a) tall plants, like pine trees
   (b) beer cans and a lot of trash
   (c) small, low-lying plants
   (d) nothing, until you walked over the first dune in back of the beach

10. Is it true that the beach tends to build up in the summer months and wear down in the winter? Explain.

11. If a dune area was destroyed by natural causes, do you think man should restore it or let nature perform the process alone?

12. Of the following measurements taken on a sandy beach, which one would probably show the least variation during the course of a day?

   (a) temperature
   (b) rainfall
   (c) wind velocity
   (d) amount of sunlight

POST-TEST

1. Briefly discuss some of the major factors which affect the growth of plants on the beach.

2. Define "profile" and how it is used in the study of beaches and sand dunes.

3. Why is it that some beach plants have a long root system?

4. What is the basis for determining the water content in sand?

5. What effect might erosion have on a relatively stable sand dune?

6. How does the "drift-line" affect the growth of a dune?

7. What is the major source of beach sand and what transports it back onto the dune?

8. To what three families do most beach plants belong? What are the common names of these families?

9. Of the four types of dunes classified, which would you consider to be the youngest type and which would be the oldest form?

10. How are quadrats used in the study of vegetation on a beach or dune?

11. Quadrat studies are tedious and time-consuming, but how may their accuracy be improved?

12. Which one of the transects is "one-dimensional" and what are some of its advantages when used?

13. In one of the measurement activities, a comparative temperature was suggested to be included in the study. What could you tell from the results of the comparison regarding the presence or absence of dune vegetation?

14. What would you consider to be the ecological significance of finding the frequency of some plant on a beach?

15. Do you feel that the measurements described in the learning activity give a good ecological picture of a beach and dune area? If not, what other factors or measurements should have been included and why?

LEARNING ACTIVITY #1

Whether one is dealing with climax communities or with a community at some other stage of ecological succession, it is important to be able to describe the communities in such a way that they can be compared with similar communities elsewhere. One also needs to know the extent of variation within any given community. (Chapman, 1964)

CONCEPT: The size and shape of a sand dune affect its ecology.

In any study of the seashore and in this case, the sand dunes, the student should prepare a beach profile. This is nothing more than measuring the horizontal and vertical distances across the sand dune. The procedure is quite simple and requires very little time.

The profile can be plotted by the use of two wooden rods as follows:

"(1) Cut two wooden rods five feet long and one inch by one inch in cross section. Cut large notches at one foot intervals or paint bright color one inch bands at each interval. Nail a small wooden plate about four inches square on the bottom. These rods are held vertically one rod length (5') apart in a line extended across the beach.

"(2) The observer holding the landward rod aligns his eye with the top of the seaward rod and the horizon , then reads and records the distance down from the top of his own rod to the point that is intersected by his own line of sight. Based on the assumption that the line of sight is level, this distance is the measure of the distance in elevation of the beach at two points five feet apart.

"(3) To continue the profile, move one of the rods to a point five feet on the opposite side of the other rod, and take a second reading. Continue to the water's edge.

"(4) Note the differences in elevation as + or - according to whether the leading rod is lower or higher than the sighting one. Differences in elevation are summed up and plotted against the horizontal distance to construct the whole profile." (Smith, 1966, pp. 240-41)

Additional directions:

(a) Profile should begin at the high water mark; this can be determined from tide tables and other charts or marine maps. Start profile on the beach and work inland across the dune area.

(b) Take careful notes when doing this profile so they can be accurately transferred to a graph and correctly plotted.

(c) The profile may be done in groups with an equal division of labor - two students making readings with the wooden poles and at least one student recording the data.

(d) Each student should construct a graphic profile of the beach and dune area as shown on the following page.

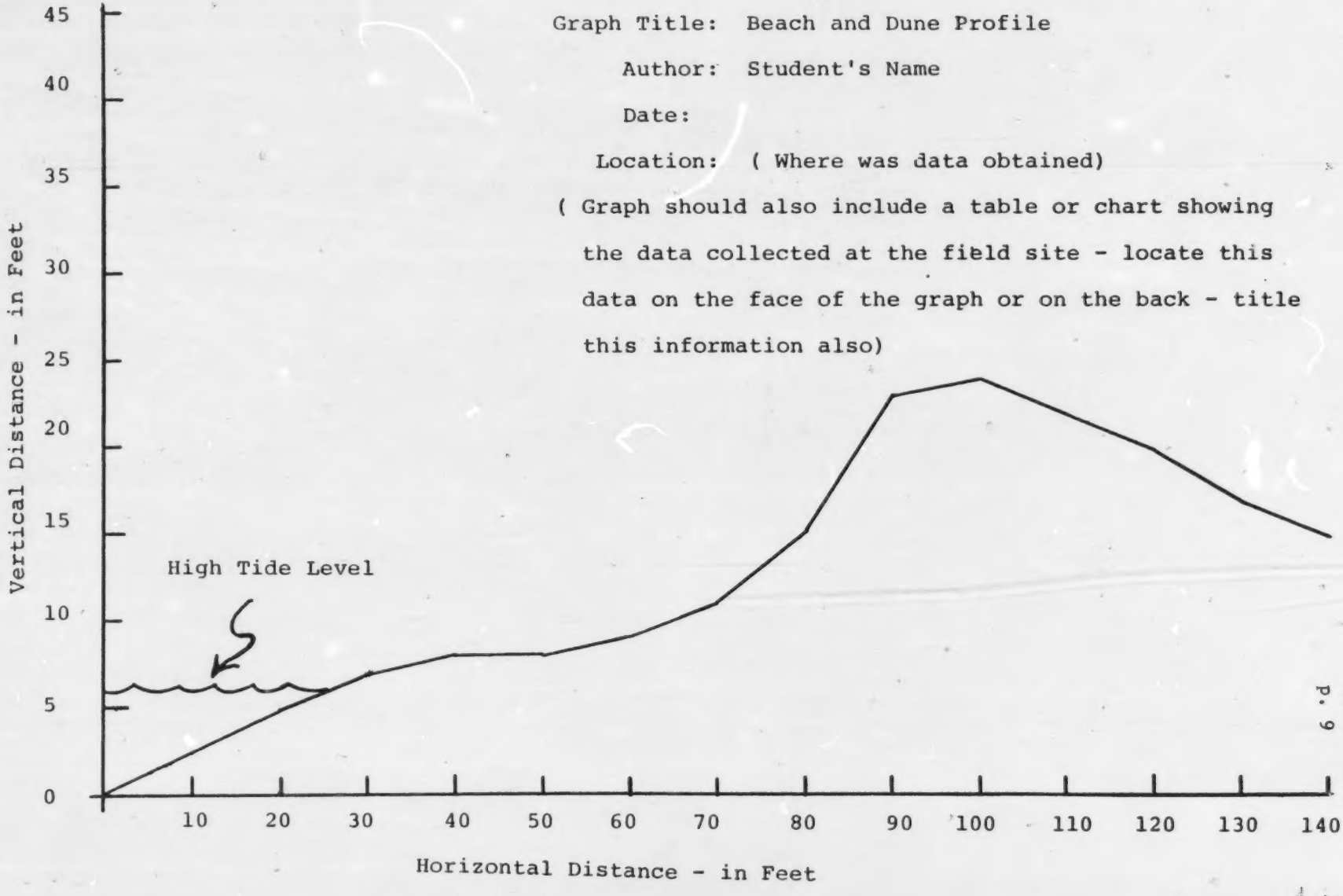
Now that you have some idea as to the shape of the beach and dune environment, let's turn our attention to some important aspects dealing with all that sand you've been walking over. One important ecological factor which readily shows itself on the dune is the presence (and absence) of water within the sand. The availability of water has a direct bearing on the kind of plants and their numbers which grow on the dune. A study of these plants will come later, but for now let's check the water content of the sand.

LEARNING ACTIVITY #2

"Since sand dunes can be regarded as edaphically dry habitats, the moisture content of the dunes and also the behaviour of the water table is of paramount interest. Digging down into the sand of maritime dunes soon shows that the subsurface layers are moist, this moisture being derived mainly from the process of internal dew formation as well as from rain. The dry character of the habitat is therefore more apparent than real, and provided plants can rapidly put out long roots water should not be a major limiting factor. Indeed, it seems evident that on young dunes it is the mobility of the sand and possible damage from sea spray that are more restrictive." (Chapman, 1964, p. 168)

CONCEPT: Moisture content of the substratum affects plant growth.

Soil (sand) moisture is of fundamental importance to plant organisms and no environmental study would be complete without measurements of this factor. This activity requires both field and lab work and can easily be incorporated into the profile activity.



I.B.3.b.(1)
(Mar. 2.4)
p. 9

Procedure is as follows:

(1) Take a sample of sand (perhaps a juice can full) at the surface, at 1 inch, 6 inches, and at 12 inches beneath the surface. The best method for taking samples would be to dig a hole one foot deep. Collect your sample from the vertical side of the hole. A sample should also be taken at the deepest point of a root system of a dominant plant on the dune.

(2) Put all samples in plastic bags and mark them accordingly with a felt-tip pen or grease pencil (mark depth and location). Marking them beforehand will decrease your collection time.

(3) Weigh drying dishes first. Weigh samples (without plastic bags) in the porcelain drying dishes. Be sure to subtract the weight of the dish from the total weight of sand sample + the dish to get weight of sample. Weigh samples carefully in grams and do not exceed the accuracy limits of the scale.

(4) The weighed samples should now be dried in a suitable oven.

(5) After several hours, remove samples, cool, then weigh dish + sand. Return them to the oven for additional drying. When samples no longer show any variation in weight, they can be considered dry.

(6) The difference in the initial weight of the sand + dish and the dried sample gives you the amount of moisture present in grams per weight of a given amount of sand.

Additional directions:

(a) Data from this activity can be plotted on graph paper as a line or bar graph.* If several locations were sampled, they might be included on the same graph - using a different color line for each location. The chart form of showing data should also be used here. (Note directions from previous Learning Activity.)

* Consult your teacher for directions on the construction of a bar (or line) graph.

LEARNING ACTIVITY #3

There are numerous methods of analyzing vegetation occupying a particular location. Several specific measuring or sampling procedures will be discussed in the next few pages regarding the plant life on the dune. These are but a few of the methods available. You will be working with one or more methods of sampling a population.

CONCEPT: The plant life on the sand dune is an intrinsic part of its ecosystem.

The Quadrat Method

An arbitrary area (quadrat) is chosen for study. Although a quadrat is usually rectangular, a circular hula hoop is an ideal sampling tool. The hoop should be tossed at random and the sample taken where it falls. The area of the hula hoop can be easily calculated (πr^2). The area of the dune can be computed by measuring two sides if it is rectangular.

Arriving at a meaningful number of sample units can be a problem. The teacher must use his judgment after appraising the dune. The number of samples needed for any degree of accuracy can be determined by statistical methods. There are various kinds of quadrats depending on the purpose of the study. Some common ones are listed below.

1. List quadrat. All organisms sampled in community are listed by name.
2. Count quadrat. The numbers of each species encountered are recorded. Forest surveys and deer-browse studies fall into this category.
3. Cover quadrat. Percentage of area covered is recorded. Might be used in studies of dunes or forest fires.
4. Chart quadrat. A map of a quadrat that is drawn to scale to show location of individual plants and provide an overall picture. (From Smith, 1966, p. 612)

"For statistically reliable estimates, the plots must be randomized. This is rather easily done. On an area photo or map of the study area, lay out a series of grid lines. Number the lines on a horizontal and vertical axis. Write the numbers of the vertical and horizontal grid lines on small squares of paper, place in two separate bowls, mix, and draw out pairs of numbers. Then locate the position of each plot by plotting the point given by the paired numbers.

"The quadrat method has its advantages and disadvantages. It is a popular method, easily employed. If the individual organisms are randomly distributed, then the accuracy of the sample and the estimate of the density depend upon the size of the sample. But since individuals seldom are randomly dispersed, the accuracy of the quadrat sampling may be low, unless a great number of plots are involved. The quadrat method is tedious and time consuming." (Smith, 1966, pp. 612-13)

Transects

"The transect is a cross section of an area used for recording, mapping, or studying vegetation. It may be a belt, a strip, or only a line on which intercepts by types of vegetation are marked off. Because of its continuity through the study area, the transect can be used to relate changes in vegetation along the line or strip with changes in the environment. As a sample unit, the measurements within a transect are pooled, and each transect is treated as a single observation in the sample." (Smith, 1966, p. 613)

The Belt Transect

"The belt transect can be set up as follows:

1. Determine the total area of the site to be sampled; then divide by 5 or 10 to obtain the total sample area.

2. Lay out a series of belt transects of a predetermined width and length, sufficient to embrace the area to be sampled. Then divide the belts into equal-sized segments. These are sometimes called quadrats or plots, but they differ from true quadrats in that each represents an observational unit rather than a sampling unit.

3. The vegetation in each unit is measured for some attribute, depending upon the problem at hand: abundance, sociability, frequency, stem count, etc." (Smith, 1966, p. 613)

(a) "abundance = $\frac{\text{Total no. of individual plants}}{\text{No. of occupied quadrats}}$ X 100 $\frac{\text{density}}{\text{frequency}}$

(b) frequency = $\frac{\text{No. of occupied quadrats}}{\text{Total no. of quadrats}}$ X 100 " (Chapman, 1964, pp.7-8)

(c) sociability = whether the plant grows singly, in clumps, mats, etc.

TABLE	BRAUN-BLANQUET'S SOCIABILITY CLASSES
Class 1	Shoots growing singly
Class 2	Scattered groups or tufts of plants
Class 3	Small, scattered patches or cushions
Class 4	Large patches or broken mats
Class 5	Very large mats or stands or nearly pure populations that almost blanket the area

(From Smith, 1966, p. 620)

The Line-Intercept Method

"The line transect is one-dimensional. It consists of taking observations on a line or lines laid out randomly or systematically over the study area.

1. Stretch a metric steel tape or steel chain between two stakes 33.5 meters, or one chain, apart.

2. Consider the line to be a belt one cm wide extending along one side of the tape.

3. Move along the line and record for each plant species found the distance it covers along the line transect:

   (a) For grasses, rosettes, and dicot herbs, measure the distance along the line at ground level.

   (b) For shrubs and tall dicot herbs, measure the "shadow," or distance covered by a downward projection of the foliage above.

4. Twenty to thirty such lines are usually sufficient.

The data can be summarized as follows:

1. The number of times each individual species appears along the line.

2. The percentage of occurrence for each species in relation to the total.

3. Total linear distance in centimeters of each species along the belt.

4. The total distance of intercept by all species per 30-meter line." (Smith, 1966, p. 614)

"This method is rapid, objective, and relatively accurate. The area may be determined directly from recorded observations. The lines can be randomly placed and replicated to obtain the desired precision. The method is well adapted for measuring changes in vegetation if the ends of the lines are well marked. Generally it is more accurate in mixed plant communities than quadrat sampling and is especially well suited for measuring low vegetation. On the debit side, the method is not well adapted for estimating frequency or abundance, since the probability of an individual being sampled is proportional to its size. Nor is it suited where vegetation types are intermingled and boundaries indistinct." (Smith, 1966, p.614)

Before you proceed further, a few review questions.

1. Which of the four kinds of quadrats lists the organisms found in that quadrat by name only?

2. Which of the four quadrats provides an overall picture of the area?

3. What are some disadvantages of the quadrat method?

4. In describing the belt transect, what is meant by "sociability?"

5. Which of the four quadrat types would you think is best suited to use on the sand dune? Explain.

Transect Study - Team Project

Each team should select a transect area somewhere in a sand dune area. This area should be easily accessible and not too far from a phone or a water source. The transect should extend from the beach face, or berm, back across the beach, through the dunes behind the beach for some 200-250 meters.

1. Make a belt transect and record all plants found 1/2 meter on each side of the line used to mark the transect. Consider 1 meter by by 10 meters a single quadrat.

   (a) For each quadrat of the transect, determine the frequency of the 3 most dominant species. Do the same for the relative

$$\text{Relative Density} = \frac{\text{No. of individuals of species}}{\text{No. of individuals of all species}} \times 100$$

   density of the three plant species and give the percentage of cover for each quadrat.

COVERAGE CLASSES

Class	Coverage, in per cent
+	Less than 1%
1	1 - 5
2	6 - 25
3	26 - 50
4	51 - 75
5	7[illegible] - 100 (Smith, 1966, p. 620)

2. Record the depth and lateral extension of the root system of one of the dominant plant species in each quadrat. Do not repeat the process if dominant type is the same in several adjoining sections. You should record the size of the plant above ground as well. Determine temperature (in Centigrade) of sand on the surface, in shade of the plant, and at several points down the root system. These levels should be about the same throughout the transect survey.

3. Construct a temperature gradient graph across the dune transect. Temperatures should be taken at the same time (noon) on both a sunny and cloudy day. This will allow for a comparison of temperatures. You should record temperatures at five feet above the sand surface, one foot above, on the surface, 1 inch below, 2 inches below, and at 5 inches below the sand surface.

4. Present as complete a list as possible of all plants found in the transect. You will probably need the aid of your teacher for this, though there are several good plant books available to help in this identification of the beach and dune plants. The slides included in this learning activity will give some idea as to what to look for on the dune. (This project is optional.)

5. All data collected should now be compiled into a report, including graphs, charts, tables, etc. A photographic summary is also highly recommended for this final report.

COMMENTS ON COLOR SLIDES

21. Shows an example of a sandy beach, the starting point for several of the measurement activities.

22. Shows the dunes that may occur immediately behind the beach.

23. Marram or beach grass growing on the dune.

24. Dune erosion exposing the extensive root systems of the marram or beach grass.

25. This is in back of the dunes where the sand approaches a low-lying bog and marsh area; transects to follow across such an area.

26 & 27. Show two views of an old salt-water pond, immediately behind the dune; good example of succession.

28. General view of low-lying area behind dune that shows varying vegetation and zones.

29. Typical plant of dune area - poverty grass.

30. Good example of a "blow-out" area.

31. Typical low shrubs and grasses in dunes.

32. Cranberries located in bog environment in back of dunes. They are not quite ripe.

33. Bayberry bush with gray-colored berries.

34. Shows the "fruit" of a particular rose bush that grows on the dunes.

35. View of the "trumpet creeper vine"; notice the two colors of the flower.

36. Close-up view of "Dusty Miller" plant, often found in the family garden or flower bed, has a nice flower.

37. Seawort. This shows the thick, fleshy leaves of many beach plants. Note its proximity to the beach surface.

38. Seaside goldenrod.

39. A sensitive plant - again note its proximity to the surface.

40. A shot of some very good blueberries.

41. This tree is showing its fruit - notice the variety in the shape of the leaves - Sassafrass.

42. View of dune area with a "scotch broom" plant in the foreground. This has a yellow flower when it blooms.

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QUANTITATIVE AND QUALITATIVE ANALYSIS OF PHOSPHATE IN WATER

A Learning Experience for
Coastal and Oceanic
Awareness Studies

Produced by

MARINE ENVIRONMENT CURRICULUM STUDY
MARINE ADVISORY SERVICE
UNIVERSITY OF DELAWARE

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TITLE: QUANTITATIVE AND QUALITATIVE ANALYSIS OF PHOSPHATE IN WATER

*CONCEPT: I.A.

I. The earth is a finite natural system.

A. THE PROPERTIES AND INTERACTIONS OF WATER, AIR, AND THE PHYSICAL EARTH SET THE LIMITS OF THE NATURAL SYSTEM.

**MARINE CONCEPT: 1.23

1. An abundance of water makes the earth unique in the solar system.

1.2 Water in the environment contains a variety of substances in suspension and in solution.

1.23 WATER IN NATURE USUALLY CONTAINS GASES, ORGANIC COMPOUNDS, AND MINERAL SALTS.

GRADE LEVEL: 11-12

SUBJECT: Chemistry

PERIODS: 6

AUTHOR: Cross

*From A Conceptual Scheme for Population-Environment Studies, 1973. Cost $2.50

**From Marine Environment Proposed Conceptual Scheme, 1973. No charge.

Both conceptual schemes are available from Robert W. Stegner, Population-Environment Curriculum Study, 310 Willard Hall, University of Delaware, Newark, DE 19711

R376

OBJECTIVES

This unit is designed to be incorporated into a high school advanced chemistry course. It can be used to expand and demonstrate certain chemical concepts and can be used in part or in its entirety.

During this unit, the student will:

1. Become aware of the presence of phosphate in sea water.
2. Understand the principles involved in using a spectrophotometer for quantitative analysis.
3. Observe applications of chemical concepts.

More specifically, the student will learn:

1. To recognize the basic importance of phosphates in natural water.
2. To apply a colorimetric method of analysis to the quantitative determination of phosphate in water.
3. To prepare a series of standard solutions by dilution from a more concentrated standard solution.
4. To prepare and use a standard calibration curve of absorbance versus phosphate concentration.
5. The basic principles involved in spectrophotometer analysis.
6. Beer's Law.
7. An alternative qualitative procedure for phosphate detection.

BACKGROUND

Phosphorus is a trace element in marine and fresh water. It can exist in natural waters in many different forms. These can be broadly categorized as orthophosphates, i.e., PO_4^{-3}, HPO_4^{-2}, $H_2PO_4^{-}$; condensed phosphates, i.e, pyro-, poly-, and meta- phosphate; and organic phosphorus. The orthophosphates are the forms of phosphorus most readily usable by organisms in water. The relative amounts of each orthophosphate depends on the pH of the water. Below pH 4.4, $H_2PO_4^{-}$ is almost the only form present. At pH greater than 9.4, PO_4^{-3} is the most abundant form.

Phosphorus and its various forms are very important compounds because phosphorus is essential to the growth of organisms. Condensed phosphates are used in the treatment of drinking water. Orthophosphates are used in detergents and fertilizers. Organic phosphates are found in animal digestive processes and in biological degradation of sewage. Therefore, high phosphate concentrations are found in waters where industrial and domestic wastes are released and where agricultural runoff is great.

The procedures for measuring phosphates discussed in this learning experience are specifically for orthophosphates; in order to analyze for other forms of phosphate it is necessary to convert the other forms to orthophosphates first.

QUANTITATIVE PROCEDURE FOR MEASURING ORTHOPHOSPHATES

THEORY

Detection of orthophosphates is accomplished by reacting it with ammonium molybdate to form molybdophosphoric acid, which is reduced to the intensely colored complex, molybdenum blue, by stannous chloride. The intensity of molybdenum blue is proportional to the phosphate concentration. This method is satisfactory for phosphate concentrations in the range of .01 to 6 mg/liter of P.

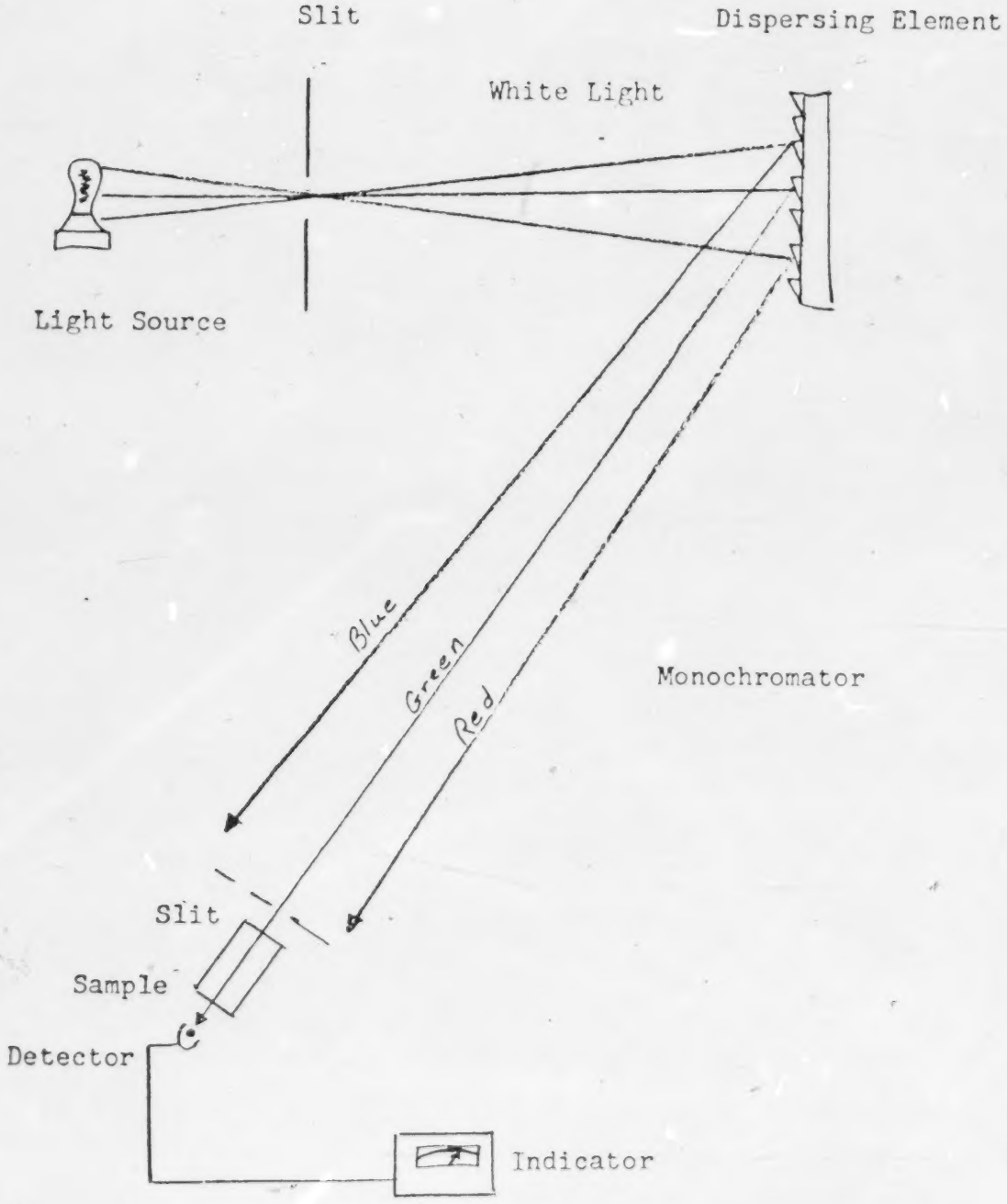
$$PO_4^{-3} + 12(NH_4)_2MoO_4 + 24H^+ \longrightarrow (NH_4)_3PO_4 \cdot 12MoO_3 + 21NH_4^+ + 12H_2O$$

$$(NH_4)_3PO_4 \cdot 12MoO_3 + Sn^{++} \longrightarrow \text{molybdenum blue} + Sn^{++}$$

The experiment described on the next few pages is based on the fact that a standard of known concentration of phosphate can be complexed to form a colored solution. This solution absorbs light proportionally to the amount of light present. Samples of unknown concentration of phosphorus are then compared by absorptivity to the standard and the amount of phosphate present can be deduced.

A spectrophotometer will be used to determine the concentration of phosphate in water. This instrument shines light of a specific wavelength on a sample solution. The light which is not absorbed by the solution is transmitted through it. The intensity of the transmitted light is then measured by phototubes in the spectrophotometer (see the diagram, p. 4) The law relating the amount of light

ESSENTIALS OF SPECTROPHOTOMETER



transmitted by a solution to the solution's concentration is the Beer-Lambert Law, better known as "Beer's Law":

$$\text{Log} \frac{I_o}{I} = A = a\,b\,C$$

where

I = intensity of monochromatic light transmitted through the solution

I_o = intensity of light transmitted through the blank

A = absorbance

b = path length (usually in cm)

a = absorptivity, a constant for a given solution and a given wavelength

C = concentration in g/liter

The law states that for a given solution, the absorbance is directly proportional to path length (the distance over which the light travels through the solution) and concentration. By measuring the intensity, the amount of light transmitted through the solution is found. The transmittance of a solution (%T) is defined as $I/I_o \times 100$.

The choice of wavelength for quantitative analysis of a particular solution is very important since the sensitivity of analysis and whether or not Beer's Law is followed depends on this choice. Wavelength selection is based primarily on the analyzed absorption spectrum. The absorption spectrum describes the absorption characteristics of the solution as a function of wavelength. Maximum sensitivity, or the largest absorptivity, a, is found at wavelengths where maximum absorption of light occurs. Similarly, minimum absorptivities are found where minimum absorption of light occurs. The Spectronic 20 is not capable of producing light with one wavelength, but rather light with wavelengths which vary by 20 nm, thus it is necessary to choose a wavelength which has an absorptivity of approximately that of the immediately adjacent wavelengths. For this reason, a wavelength corresponding to a flat portion in the absorption spectrum is generally chosen if possible.

Deviations from Beer's Law can occur for a number of reasons. For example, the spectrophotometer's wavelength variance of 20 nm , when passed through the sample can cause a deviation, particularly if the absorptivity, a, is significantly different for these varying wavelengths. This deviation usually becomes larger at higher concentrations. Chemical reactions of solute, temperature effects in some instances, stray or scattered light, fluorescence of solutes, and other factors can also cause deviations.

PROCEDURE

1. Make up the reagents before the lab period (see page 8).
2. Turn on the spectrophotometer and allow it to warm up for at least 30 minutes.
3. Calibrate with distilled water:
   a. Adjust the amplifier control knob until the needle indicates 0% T with no cuvette in machine.
   b. Select one cuvette for the blank solution (in this case distilled water). Do not interchange it with the other cuvettes. Do not handle the lower portion of the cuvette through which the light passes. Always rinse the cuvette with several portions of distilled water before taking a measurement. Place approximately 3 ml distilled water in the cuvette for analysis. Wipe the outside of the cuvette with lens paper--never use a handkerchief or towel for this purpose. Insert the cuvette into the cell holder with the index line facing you to avoid scratching. After the cuvette is seated, line up the index lines exactly.
   c. Set 100% T when the blank is in the cell holder at 690 nm.
   d. Set 0% T when no cuvette is in the cell holder at 690 nm.
4. Prepare six dilutions of the stock phosphate solution (1 ml = 50 µg PO_4-P) and a blank by pipetting 10, 20, 40, 60, 80 and 100 µg P into individual volumetric flasks. Develop the color by adding with thorough mixing 4 ml molybdate reagent and 4 ml H_2SO_4. Add 10 drops stannous chloride reagent and dilute to 50 ml with distilled water. Wait at least ten minutes for color development. For the blank, distilled

water should be substituted for the phosphate solution. To determine the volume of water to be used in place of phosphate in the blank, divide the mg by 50 mg/liter (since 1.0 ml = 50 µg P, then 1 = $\frac{50 \mu g\ P}{1.0\ ml}$)

Vol. = $\frac{\text{weight}}{\text{weight/ml}}$ = $\frac{10 \mu g}{50 \mu g/ml}$ = 0.2 ml

Vol. = " " = $\frac{20 \mu g}{50 \mu g/ml}$ = 0.4 ml

Vol. = " " = $\frac{40 \mu g}{50 \mu g/ml}$ = 0.8 ml

Vol. = " " = $\frac{60 \mu g}{50 \mu g/ml}$ = 1.2 ml

Vol. = " " = $\frac{80 \mu g}{50 \mu g/ml}$ = 1.6 ml

Vol. = " " = $\frac{100 \mu g}{50 \mu g/ml}$ = 2.0 ml

To determine the concentration of phosphate in the standard dilutions, divide the weight of phosphate by the total volume. Thus,
$\frac{10 \mu g}{50\ ml}$ = 0.2 µg/ml.

5. Determine the absorption of each of the six solutions prepared in step 5, making measurements at 705 nm, resetting 0 and 100 % T before each measurement as follows:
   a. Set 0% T when no cuvette is in the cell holder.
   b. Set 100% T when the blank is in the cell holder.
   c. Insert the cuvette containing the diluted standard. Read and record % T.
6. Plot absorbance vs. concentration on graph paper to form a standard curve.
7. Measure the absorbance at 690 nm of the two unknowns. (Make sure to develop the color as in step 4). Reset 0% T and 100% T before each measurement.
8. Take the absorbance values of the two unknowns measured in step 7. Using the standard curve plotted in step 6, determine the concentration of the phosphate in the unknowns.

APPARATUS

1. Spectrophotometer, for use at 690 nm. (Bausch & Lomb Spectronic 20 or equivalent.)
2. Acid-washed glassware. Phosphate contamination is common due to its absorption on glass surfaces. Detergents may contain phosphates, therefore all glassware should be cleaned with hot dilute HCl and rinsed well with distilled water. Once the cleaning procedure has been performed, the glassware can be reused without repeating the treatment.
   a. Pipets
   b. Volumetric flasks
   c. Cuvettes - 2
   d. Beakers
   e. Sample bottles

REAGENTS

1. Stock phosphate solution, 50 mg P/ml: Dissolve 219.5 anhydrous potassium dihydrogen phosphate (KH_2PO_4) in distilled water and dilute to 1 liter. (Approximately 300 ml per student group will be needed.)
2. Sulfuric acid solution: Cautiously add 300 ml concentrated H_2SO_4 (9M) to 600 ml distilled water, and allow to cool. When cool, add 4.0 ml concentrated HNO_3 and dilute to 1 liter.
3. Ammonium molybdate solution: 0.96% molybdate:
   a. Dissolve 25 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 175 ml distilled water. Cool.
   b. Cautiously add 280 ml concentrated H_2SO_4 to 400 ml distilled water. Cool.
   c. Add the molybdate solution to the acid and dilute to 1 liter.
4. Stannous chloride reagent: Dissolve 2.5 g of a fresh supply of $SnCl_2\cdot 2H_2O$ in 100 ml glycerol . Heat in a water bath and stir with rod to hasten dissolution. This reagent is stable and requires neither preservatives nor special storage.
5. Solutions with unknown phosphate content:
   a. Add 5 mg Tide or another similar phosphate-containing detergent to 1 liter distilled water. Lower pH to 1 with concentrated HCl or H_2SO_4. Boil in an erlenmeyer flask for 30 minutes. Cool. Readjust pH to neutrality with approximately 15M NaOH solution and readjust volume to 1 liter. (Approximately 100 ml per student group will be needed.)
   b. Add 5 mg Tide (or another similar phosphate-containing detergent) to 1 liter distilled water. Adjust pH to 7. Boil for 30 minutes and readjust volume to 1 liter.

EXAMPLE OF COLORIMETRIC ANALYSIS OF PHOSPHATE AT 890 nm

(See graph, p. 10)

Weight of P in µg	P-Concentration* µg/ml	Absorbance	%T
12	0.24	0.25	56.0
20	0.40	0.41	39.0
40	0.80	0.87	13.5
60	1.20	1.30	5.0
80	1.60	1.70	2.0
100	2.00	2.00	1.0

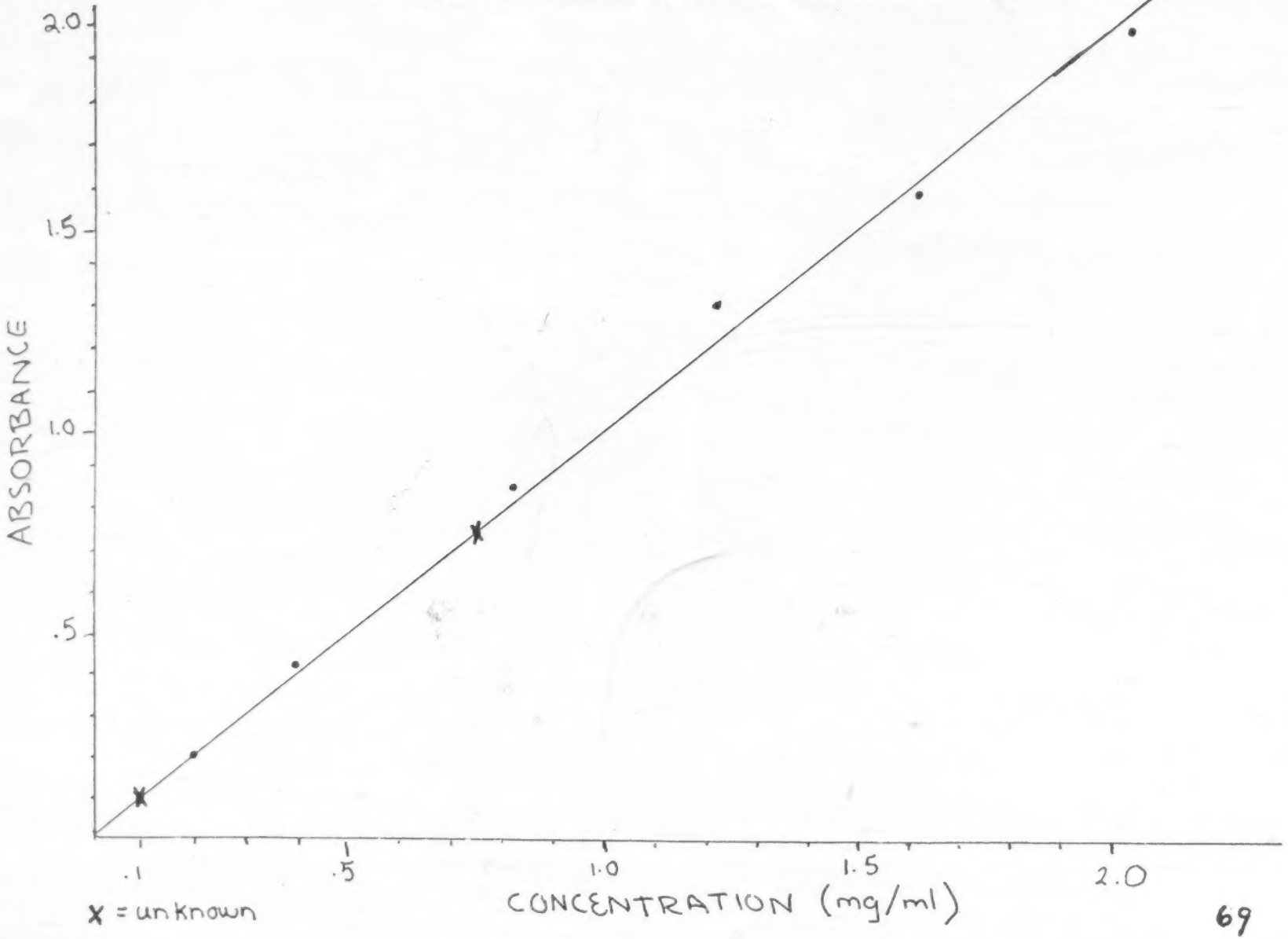
Data for Unknown Samples

Sample	Absorbance	P-Concentration µg/ml	mg/ml
a.	0.78	0.75	0.75
b.	0.10	0.10	0.10

*P-Concentration is calculated by dividing the known weight of phosphate in the standard sample by the volume of the standard, which in this case, is 50 ml.

**Since sample contains 4 g P/ml, the volume is $\frac{g\ P}{4\ \mu g/ml}$

STANDARD CALIBRATION CURVE AT 890 nm.



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QUALITATIVE PROCEDURE FOR MEASURING ORTHOPHOSPHATE

THEORY

When the orthophosphate is reacted with ammonium molybdate, ammonium phosphomolybdate is formed. The product is reduced to molybdenum blue by reaction with stannous ions.

a. $PO_4^{-3} + .12(NH_4)_2MoO_4 + 24H^+ \longrightarrow (NH_4)_3PO_4 \cdot 12MoO_3 + 21NH^+ + 12H_2O$

b. $(NH_4)_3PO_4 \cdot 12MoO_3 + Sn^{++} \longrightarrow$ (molybdenum blue) $+ Sn^{+4}$

EQUIPMENT

2 test tubes

3 eyedroppers

REAGENTS

1. Stock 0.001 M Phosphate Solution: Add 0.136 g KH_2PO_4 to distilled water making total volume of one liter.
2. Working Standard; 1.0 ppm H_2PO_4: Add 10 ml of stock solution to 990 ml distilled water.
3. Ammonium molybdate solution; Nitric Acid Reagent:
   a. Dissolve 15 g ammonium molybdate in 300 ml distilled water.
   b. Cautiously add 100 ml of a 1:1 dilution of nitric acid and saturate with ammonium nitrate. Cool.
   c. Add the molybdate solution to the acid and dilute to one liter.

PROCEDURE

1. Prepare a reference sample containing phosphate ions by placing 20 drops of working standard in Tube 1.
2. Place 20 drops of the water sample in Tube 2. The water sample may be obtained from the tap, a local stream, a pond, a bay, or from the unknowns made on p. of the learning experience.
3. Add 10 drops of the ammonium molybdate-nitric acid reagent to each tube.
4. Add a few crystals of stannous chloride to both tubes. A blue color should appear if orthophosphate ions are present.
5. Compare the intensity of the blue pigment in Tube 2 with that in Tube 1 (1 ppm).

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